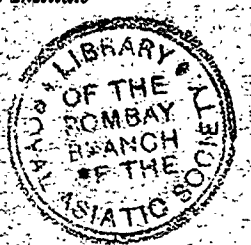
Report of the Haffkine Institute

For the Year 1938

BY

LT.-COL. S. S. SOKHEY, I.M.S.
Director, Haffkine Institute



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REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1938.

PART I.

STAFF.

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Mr. R. D. Panwalkar, A.M.	..	Artist and Photographer.
Mr. M. V. Kamat, B.A.	..	Librarian.
Mr. S. Y. Nabar	..	Packing.

Junior Staff, Subordinates and Inferior Establishment number 109.

GENERAL REVIEW.

In general the activities of the Institute remained the same as in previous years. The production of large quantities of prophylactic vaccines, mainly Haffkine Plague vaccine, cholera vaccine, typhoid vaccine and antirabic vaccine, and the diagnostic work for the hospitals largely monopolised its limited resources in funds and personnel. An idea of the work involved in the production of vaccines alone can be gained from the fact that 3,411,258 cc. (i.e., 1,137,086 doses of plague vaccine, 608,506 cc. of cholera vaccine, 38,269 cc. of typhoid vaccine and 733,530 cc. of 5 per cent. antirabic vaccine were issued during the year. Despite the pressure of routine duties most members of the staff were enabled by the generous aid of the Indian Research Fund Association in funds and personnel to carry out valuable researches detailed elsewhere in this report. For plague and pharmacological researches the Association contributed about Rs. 50,000. Two voluntary workers and a Lady Tata Memorial Scholar also contributed to the research activities of the Institute.

During the year Government sanctioned a valuable addition to the staff. Advantage was taken of the retirement of Rao Bahadur Chitre to engage an Entomologist in his place. The services of a noted Indian Entomologist, Dr. M. Sharif, who is an authority on fleas, were secured and a department of Entomology organised. Since insects play an important role in the spread of disease in the tropics this new department should prove of great value to the health organisation of the Province, if the staff could be strengthened to enable the Institute to undertake field investigations. The time of the present staff is fully occupied by laboratory bench work and it does not permit the Institute, the only medical research organisation of the Province, to play that role in the health organisation of the Province which it should by conducting field and other studies on the urgent health problems of the people in close association with the Public Health Department. In the 1924 report the then Director strongly stressed the value of such an association and advocated the organisation of a mobile unit. The need of such a unit is greater than ever, and it is hoped the staff of the Institute will be strengthened to permit of such work being undertaken. How valuable such an association can be was demonstrated by the enquiry undertaken during the year by the Institute into the outbreak of typhoid fever in the City, in association with Municipal Health authorities. Results of the enquiry are given elsewhere in this report.

Plague Department.

(1) *Antiplague Serum.*—A small outbreak of plague at Bettiah, Bihar permitted a field trial of the antiplague serum prepared at the Institute to be carried out. A temporary hospital was erected at the place and other facilities provided by the Bettiah Raj and the Bihar Government for which our grateful thanks are due.

The trial lasted from the middle of January to the end of April 1938. 124 cases of plague were dealt with. Every alternate admission was

treated with serum ; others were treated with intravenous injections of iodine solution. No selection of any type was made, but during the third week of the trial all patients admitted were treated with serum. Thus 69 patients were treated with serum, and of these 19 died of plague, giving a percentage mortality of 27 per cent. Of the 55 cases treated with iodine injections, 36 died, giving a percentage mortality of 65 per cent. If to these figures we add the figures of a previous trial conducted at Hyderabad with the same serum, we get 94 patients treated with serum, with 24 deaths, and percentage mortality of 25·5. From among 80 controls, there were 50 deaths giving a percentage mortality of 62·5. Clinically the administration of the serum produced very striking results ; the general condition of the patients improved very rapidly with the disappearance of toxic symptoms. These results show that we have obtained a serum of a high curative value. The preparation of this serum was made possible by the exact biological methods worked out in this Institute and described elsewhere in this report. Animal experiments were designed which closely reproduced the conditions which severe cases of plague present, and serum was tested on such animals. These experiments yielded a clear idea as to the conditions under which the best serum could be obtained. The full significance of these results can be realised only when they are compared with the results obtained with other antiplague sera tried since the beginning of the present pandemic. None of these sera reduced the case mortality below 60 per cent.

(2) *Chemotherapy of Plague.*—Some of the sulphanilamide compounds which have proved to be so valuable in the treatment of streptococcal infection were tested for their curative value in plague infection. For this purpose animal experimental conditions were designed to produce disease approximating to the actual severe cases of plague. The compounds tested, Prontosil and M and B, 693, showed little or no curative power. Further work is proceeding.

(3) *Plague Vaccine.*—No changes were introduced in the preparation of the plague vaccine during the year. Though the recent researches conducted at the Institute have thrown a flood of light on the problem of the preparation of a plague vaccine, there are still some obscure points to be solved. Exact determinations are being made to compare the protective powers of agar and broth vaccines with a view to see which of the two methods gives the best chance of producing vaccines of uniformly high protective power. Experiments are also being conducted to increase the growth of organism in broth cultures, as the protective power of a broth vaccine incubated at a suitable temperature depends on the number of the organisms growing in the medium.

(4) *Serology of Plague.*—A great deal of work has been conducted to elucidate the phenomena of agglutinin and precipitin reaction and their relation to active immunity in plague, but the results are still very divergent and more work will have to be carried out before any worthwhile conclusion can be drawn.

Enquiry into the Outbreak of Typhoid Fever in the City of Bombay.

During the year there was a sudden increase in the incidence of typhoid fever in the City and the Institute was asked to investigate into the sources and mode of this outbreak in collaboration with the Municipal Health authorities. The Institute assumed responsibility for (1) laboratory diagnosis of cases by sending out an officer to take samples of blood and other material of cases on receipt of a call from any medical practitioner or a hospital and (2) investigation into the source or sources of infection and the mode of spread of the disease.

Cases.—From the 25th of June to the end of the year, the Institute examined 398 cases, out of which 234 proved to be cases of typhoid fever and 16 cases of paratyphoid A. The fact which requires to be stressed here is that this laboratory aid in the diagnosis of cases, given absolutely free of charge to medical practitioners, improved considerably the situation regarding the notification of this disease in the City. For the five year period previous to the present enquiry 1,959 attacks and 1,349 deaths had been notified from typhoid. These figures give a very improbable case mortality of 69 per cent. while the present enquiry yields among the definitely diagnosed cases, the very likely figure of 9·3 per cent. case mortality, showing that in previous years roughly 1 attack out of every 6 occurring in the City was notified. It was obvious that the medical practitioners appreciated the help given to them ; they were willing to make use of it, leading to a much higher percentage of notification. It is very gratifying to note that Government is devising a permanent scheme to make available to medical practitioners such laboratory aid in the diagnosis of their cases, free of charge.

Sources and mode of spread.—Though the water supply of the City was not found to be up to the required standard of purity, it was not held responsible for the typhoid outbreak. Largest number of cases occurred in unsewered parts of the City where basket privies were in use. Some sewage from the areas was allowed to flow into the open drains. This, coupled with a great increase in the number of flies in the area, explained part of the trouble. Direct contact due to overcrowding in tenement houses and the refusal of patients to go to the infectious diseases hospital was another factor in the spread of the disease. No articles of food examined were incriminated, but the carrier problem could not be tackled for lack of co-operation on the part of the public.

Department of Pharmacology.

The Department continued its studies on synthetic anti-malarial drugs, plasmoquine and atebrin. The observation reported last year that placenta filtered out plasmoquine and prevented large quantities of the drug from entering the foetal circulation, was further confirmed by the actual measurement of the concentration of plasmoquine in the maternal and foetal blood simultaneously after giving large doses of the drug to the mother. It was further shown that therapeutic

doses of plasmoquine given to pregnant experimental animals, did not in any way affect the litters given by the animals. These studies show that this drug can be safely used during pregnancy.

The fate of plasmoquine and atebrin in the body has been studied. Plasmoquine is largely destroyed by the liver and atebrin was retained by reticulo-endothelial cells. A very important observation regarding the formation of acetylcholine was made. It was found that chiefly nerve cells were responsible for its formation.

Dr. Inderjit Singh, a young promising research worker, posted to the Department during the year by the Indian Research Fund Association, conducted some important experiments elucidating the interaction of ions, drugs and electrical stimulation on the contraction of unstriated muscle.

Department of Biochemistry.

Some studies conducted by the department had shown that the average hæmoglobin content of blood of Indian normal subjects tallied exactly with similar averages worked out in America for white American subjects. These averages were 15·37 g. and 12·99 g. per 100 cc. of blood for men and women respectively. This observation suggested the possible existence of a hæmoglobin constant. 19 subjects have so far been examined and attempt is being made to find if there is a definite ratio between hæmoglobin content and some such unit as body surface.

The power of the kidney to eliminate urea from the blood stream is employed as a measure of renal function for clinical purpose. Moller, MacIntosh and Van Slykes blood urea clearance test worked out on American subjects is usually employed for the purpose. It was noticed in the course of clinical work that in the case of normal Indian subjects while the blood urea content is within the same limits as that of American subjects the urea content of urine of normal Indian subjects is about half of American subjects. This raises an interesting issue whether the formula on which the urea clearance test is based applies to Indian subjects. This subject is being studied.

Diagnostic Department and the Department of Public Health Chemistry and Bacteriology.

In addition to the usual routine work handled by these departments which amounted to an examination of over 10,000 specimens during the year, these departments had to carry out the major part of the work in connection with the inquiry into the outbreak of typhoid fever in the City of Bombay. Not only that, but the departments had also to send out an officer to take blood and other samples from patients on the request of any medical practitioner. Over 600 such visits were paid during the months of July to December.

Antirabic Department.

Four more antirabic treatment centres were opened during the year, making a total of 87 in the Province and the neighbouring areas. The opening of these centres is a real boon to the public. These centres

enable the public to get the antirabic treatment without having to make unnecessarily long journeys.

Demand for the antirabic treatments showed a further rise during the year. 11,479 treatments were issued as compared with 9,715 treatments issued last year.

SOCIAL LIFE AT THE INSTITUTE.

The Critics Club organised in 1924, plays an important role in the collective life of the Institute. At its regular meetings the members of the staff present the scientific work being carried out by them and invite discussion. These discussions stimulate and maintain general interest in all the work being carried out in the different departments of the Institute. The club also provides social intercourse among the members of the staff, by holding social functions.

The Critics Tennis Club and the Haffkine Institute Sports Club have largely increased their membership. Keenness of the staff for sports is shown by increased attendances at games. The games of Tennis, Cricket, Hockey, Football and Badminton are all well patronised. There were good entries for the Mackie and Sokhey Tennis Cups and well contested games made the tournaments very enjoyable and the success of the tournaments was largely due to the excellent arrangements made by the Secretary, Lt. Maurice.

Haffkine Institute Co-operative Credit Society, Ltd.—This is the third year of the Society. The Society is showing excellent progress. To encourage thrift the Society has started insuring the lives of its members with the Bombay Provincial Co-operative Insurance Society, Ltd., and has undertaken to give loans free of interest to be used for paying premia.

SECTION I.

VACCINE AND SERUM DEPARTMENT.

A. *Haffkine's Plague Vaccine.*

1,137,086 doses (i.e., 3,411,258 cc.) of plague vaccine were issued during the year against 1,324,337 doses in the previous year.

The number of doses of plague vaccine despatched monthly during the years 1937 and 1938 are detailed below :—

	1937	1938
January	132,937	190,419
February	141,074	78,931
March	116,278	109,280
April	91,915	6,170
May	14,009	5,390
June	18,601	570
July	24,423	46,974
August	219,169	84,459
September	206,912	234,889
October	48,629	100,027
November	203,860	99,341
December	106,530	180,636
	1,324,337	1,137,086

From the opening of the laboratory to the end of 1938 a total of 43,371,794 doses have been issued. The output of the vaccine in quinquennial periods has been as follows :—

Years.	Doses.
1897–1901	2,867,041
1902–1906	3,455,537
1907–1911	3,584,262
1912–1916	4,567,366
1917–1921	6,667,894
1922–1926	7,202,636
1927–1931	5,929,243
1932–1936	6,636,392
1937–1938	2,461,423
	43,371,794

The geographical distribution of the vaccine in India and to places outside India during the years 1937 and 1938 is given below :—

	Number of doses.	
	1937	1938
British India.		
Bombay Province	106,867	59,436
Madras Province	213,433	139,802
Bengal, Bihar and Assam	40,924	30,283
United Provinces of Agra and Oudh	278,183	231,180
Punjab	5,628	3,098
North West Frontier Province	135	292
Central Provinces and Berar	52,060	39,812
Delhi Province	282	489
Sind	17,339	8
	714,851	504,400

	Number of doses.	
	1937.	1938.
Indian States.		
Southern Maratha States	21,566	17,934
Hyderabad, Deccan	256,696	124,940
Mysore	212,972	257,730
Patiala	156	....
Kashmere	....	1
Jaipur	103	....
Jodhpur	48	....
Bhopal	392	2,400
Indore	8	16
Cochin	1,200	504
Rewa	....	1,428
Baroda	1,488	1
Gwalior	4,032	....
Sawantwadi	304	....
Rajkot	12	....
Junagad	400	....
Benares State	8,155	3,504
Jammu	....	1,800
Travancore	....	504
Bikaner	....	504
	507,532	411,266
Foreign Countries.		
Burma	90,816	219,630
Ceylon	400	....
Aden	5,040	1,008
Federated Malay States	1,062	590
Cairo, Egypt	1,400	....
Iraq	3,236	....
Portuguese Government	....	192
	101,954	221,420
Total number of doses ..	1,324,337	1,137,086

B. *Cholera Vaccine.*

The active campaign against the spread of cholera in this Province initiated by the Public Health Department a few years ago has again made large demands for the supply of cholera vaccine and over 6 lakhs of doses were issued during the year.

Cholera vaccine issued month by month from 1936 *to* 1938.

Month.	1936 in cc.	1937 in cc.	1938 in cc.
January	11,191	7,958	4,165
February	12,801	5,650	2,352
March	4,553	11,688	8,421
April	4,341	33,655	924
May	21,364	87,339	31,120
June	192,563	41,235	235,938
July	118,183	207,945	96,692
August	92,487	135,100	85,755
September	64,173	49,360	93,065
October	55,370	19,453	20,019
November	33,330	11,168	15,925
December	95,265	30,427	14,130
Total ..	705,621	640,978	608,506

C. *Other Vaccines.*

Statement of vaccines issued monthly during the year 1938.

Month.	Mixed T. A. B. Vaccine in cc.	Meningococcal Vaccine in cc.
January	328	5
February	665	310
March	686	125
April	295	....
May	2,338	....
June	11,439	....
July	8,706	....
August	9,496	....
September	2,238	75
October	1,165	....
November	247	....
December	666	50
Total ..	38,269	565

D. *Sera.*

During the year under report the following sera were issued by the Institute :—

		Ampoules.
1.	Anti-Tetanic Serum (Prophylactic) 1,500 International Units, 1½ cc.	7,487
2.	Anti-Tetanic Serum (Curative) 20,000 International Units, 10 cc.	1,641
3.	Anti-Diphtheria Serum (Prophylactic) 1,000 International Units, 1 cc.	111
4.	Anti-Diphtheria Serum (Curative) 10,000 International Units, 10 cc.	233
5.	Anti-Dysenteric Serum 8,000 International Units, 20 cc.	924
6.	Anti-Streptococcus Serum Polyvalent, 10 cc. ..	457
7.	Anti-Meningococcus Serum, 10 cc.	211
8.	Anti-Gas Gangrene Serum, 10 cc.	954
9.	Normal Horse Serum 5 cc.	48
10.	Normal Horse Serum, 10 cc.	365

SECTION II.

Epidemiology of Plague.

This department carried out a number of routine duties and researches during the year. For researches see Part II of this report.

A. *Training of Health Personnel.*

1. At the request of the Director of Public Health, Travancore, Mr. George Ooman was given a month's course of training in anti-plague measures.

2. Dr. S. Dakshinamurthy, M.B.B.S., D.P.H., D.T.M. & H., Municipal and Port Health Officer, Vizagapatam, was given a short course of training in plague work.

3. The 1938 class of Sanitary Inspectors, divided into four batches, was given a short course of demonstrations and lectures in the subject of rat examination and rat and flea destruction.

B. *Examination of Bombay City Rats.*

The examination of rats and other rodents of Bombay City, sent by the Executive Health Officer of Bombay Municipality, for evidence of plague, was continued during the year. The Municipality maintains a permanent establishment for catching rats and about 2,000 rats are caught every night in the City and they are marked with the locality from where they are caught. Out of these rats, those which arrive dead at the Institute, are post-mortemed and examined for plague. In doubtful cases smears and cultures are made.

This study besides keeping the Municipality informed of any plague epizootic in any particular part of the City, is providing valuable information on the subject of plague epidemiology.

Tables summarising the monthly figures of rats received at this Institute and the number out of them that were examined are given below. No plague infected rat was found during the year and no human case of plague occurred in the City during the year.

Table I.

Rats received at the Institute from the Municipality during 1938.

Months.	Alive.	Dead.	Total.
January	32,110	33,102	65,212
February	28,465	29,421	57,886
March	32,265	28,084	60,349
April	27,999	25,399	53,398
May	25,736	25,238	50,974
June	30,496	22,439	52,935
July	34,286	23,658	57,944
August	35,107	22,947	58,054
September	39,249	23,309	62,558
October	47,471	24,691	72,162
November	38,401	28,918	67,319
December	38,930	36,482	75,412
Total ..	410,515	323,688	734,203

TABLE II.

Examination of Dead Rats for Plague during 1938.

Months.	Dead rats examined.	Found plague infected.	
		Rattus norvegicus.	Rattus rattus.
January	19,566		
February	17,874		
March	17,637		
April	12,879		
May	14,968		
June	12,358	*Nil.*	*Nil.*
July	14,547		
August	12,780		
September	13,892		
October	13,118		
November	19,536		
December	18,115		
Total ..	187,270		

TABLE III.

Relation of Human Plague Deaths to Plague Deaths among Rats during 1938.

Months.	Human plague deaths.	Rats examined.	Plague infected rats.
January	0	19,566	0
February	0	17,874	0
March	0	17,637	0
April	0	12,879	0
May	0	14,968	0
June	0	12,358	0
July	0	14,547	0
August	0	12,780	0
September	0	13,892	0
October	0	13,118	0
November	0	19,536	0
December	0	18,115	0
Total ..	0	187,270	0

TABLE

Table giving the monthly figures of rats trapped alive by Municipal

Monthly.

Species.	January.		February.		March.		April.		May.		June.		July.	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
Rattus rattus ..	5,309	20·7	4,797	20·7	6,538	24·1	3,872	18·7	4,450	20·8	5,191	20·8	6,900	23·5
Rattus norvegicus..	6,915	27·0	6,004	25·8	6,716	24·8	5,514	26·6	5,383	25·2	6,534	26·2	7,548	25·5
Gunomys varius ..	8,030	31·4	6,664	28·7	8,120	29·9	5,932	28·6	5,798	27·1	6,395	25·6	7,347	24·8
Bandicota indica ..	267	1·0	234	1·0	238	0·9	181	0·8	264	1·2	356	1·4	515	1·8
Mus musculis ..	1,421	5·6	1,445	6·2	1,342	5·0	1,131	5·4	1,245	5·8	1,298	5·2	1,945	6·5
Crocidura coerulea	1,398	5·5	1,422	6·1	1,634	6·0	1,587	7·6	1,383	6·5	2,077	8·3	2,180	7·4
*Unidentifiable ..	2,248	8·8	2,667	11·5	2,535	9·3	2,546	12·3	2,863	13·4	3,113	12·5	3,114	10·5
†Total..	25,588	100·0	23,233	100·0	27,123	100·0	20,763	100·0	21,386	100·0	24,964	100·0	29,549	100·0

*Rats included under this head though reported to have been caught alive, were

†These totals do not agree with the totals of live rats in Table I because rats

IV.

Corporation, Bombay, during 1938—*classified according to species.*

	August.		September.		October.		November.		December.		For whole period.	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
	6,072	21·4	8,123	24·7	12,438	34·6	9,849	30·9	7,388	29·2	80,927	24·7
	6,651	23·4	8,861	26·9	7,704	21·4	8,656	27·2	6,776	26·8	83,262	25·4
	7,402	26·2	9,830	29·9	9,334	26·2	7,747	24·4	6,866	27·2	89,465	27·3
	433	0·1	422	1·3	406	1·2	313	0·9	311	1·2	3,940	1·2
	2,297	8·5	2,186	6·6	1,386	3·8	1,794	5·6	1,481	5·8	18,971	5·8
	2,360	8·6	1,988	6·1	1,779	4·9	1,969	6·3	1,463	5·7	21,240	6·4
	3,096	11·8	1,506	4·5	2,850	7·9	1,502	4·7	1,047	4·1	29,087	9·2
	28,311	100·0	32,916	100·0	35,897	100·0	31,830	100·0	25,332	100·0	326,892	100·0

received at the Institute dead, often mutilated and even putrid.

received on Sundays and holidays were not classified.

TABLE

Table giving the classification of rats found dead in

Monthly.

Species.	January.		February.		March.		April.		May.		June.		July.	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
Rattus rattus ..	2,874	11·2	2,464	10·7	1,992	8·9	1,382	8·2	1,334	6·6	1,123	6·8	1,328	6·8
Rattus norvegicus ..	6,823	26·6	6,705	29·0	6,388	28·5	4,572	27·1	5,084	25·3	4,293	25·9	4,754	24·6
Gunomys varius ..	6,965	27·2	5,842	25·3	5,445	24·3	3,188	18·9	4,479	22·3	3,701	22·3	3,713	19·2
Bandicota indica ..	375	1·5	387	1·7	332	1·5	143	0·9	178	0·8	151	0·9	92	0·5
Mus Musculis ..	2,529	9·9	2,476	10·8	1,730	7·7	1,490	8·8	1,302	6·5	1,201	7·3	2,110	10·9
Crocidura coerulea	1,564	6·1	1,621	7·0	1,999	8·9	1,501	8·9	2,163	10·8	2,320	13·9	2,253	11·8
Unidentifiable ..	0	0	1,273	5·5	2,160	9·7	2,124	12·6	2,599	12·9	1,884	11·4	2,572	13·3
Putrid ..	4,489	17·5	2,304	10·0	2,359	10·5	2,476	14·6	2,957	14·8	1,913	11·5	2,462	12·9
*Total ..	25,619	100·0	23,072	100·0	22,405	100·0	16,876	100·0	20,096	100·0	16,586	100·0	19,284	100·0

*These totals do not agree with the totals of dead rats in table No. 1 because

V.

the City of Bombay during 1938.

August.		September.		October.		November.		December.		For whole period.	
Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
1,533	8·9	1,456	8·1	2,189	13·4	2,942	12·2	2,728	12·3	23,345	9·8
4,045	23·7	4,753	26·2	4,094	23·7	6,381	26·9	6,215	28·3	64,107	26·5
3,024	17·8	3,503	19·3	3,244	19·3	4,572	19·2	4,790	21·7	52,466	21·7
159	0·9	183	1·1	223	1·3	265	1·1	144	0·8	2,632	1·0
2,082	12·2	2,136	11·7	1,791	10·6	3,186	13·3	2,158	9·8	24,191	10·0
2,158	12·7	2,047	11·2	1,854	11·5	1,922	8·2	1,619	7·3	23,021	9·5
1,937	11·3	1,861	10·2	1,577	9·3	2,190	9·2	2,080	9·4	22,257	9·2
2,146	12·5	2,184	12·2	1,848	10·9	2,349	9·9	2,296	10·4	29,783	12·3
17,084	100·0	18,123	100·0	16,820	100·0	23,807	100·0	22,030	100·0	241,802	100·0

rats received on Sundays and holidays were not classified.

C. *Examination of Rats from Steamers in the Bombay Harbour and from other places.*

The Health Officer of the Port of Bombay forwarded 188 rats (104 killed and 84 alive) from 15 steamers. Out of the 104 killed rats, 23 (22·1 per cent.) were Rattus rattus, 79 (76 per cent.) were Mus musculis, and 2 (1·9 per cent.) were unsuitable for identification. Of the 84 live rats, 79 (94 per cent.) were Rattus rattus, 4 (4·8 per cent.) Rattus norvegicus, and 1 (1·2 per cent.) Mus musculis. The 84 live rats yielded altogether 162 fleas, which were all Xenopsylla cheopis (69 females and 93 males). All the above rats were examined for plague and none was found plague infected.

D. *Examination of Rats from Other Sources.*

During the year 264 rats were received for examination from different towns of the province. Of these none showed any evidence of plague infection. Smears from 7 other rats were also received amongst which one showed presence of *Past. pestis.*

E. *Preparation and Supply of Exhibits on Plague and Other Diseases.*

The department prepared several exhibits during the year and supplied them to various bodies for use or for public exhibition. Specimens and smears showing lesions of plague were also supplied to different teaching institutions.

F. *The Manufacture of Approved Rat Traps.*

During the year several samples of rat traps from different manufacturers were received from the Bombay Municipality for examination and expert opinion. General assistance was given to different manufacturers in the matter of producing the right kind of rat traps.

SECTION III.

Department of Pharmacology.

The Department continued its work on the same lines as reported last year, and is still under the Indian Research Fund Association. Besides, Dr. B. K. Nandi, whose services were engaged last year the Indian Research Fund Association were also pleased to appoint Dr. Inderjit Singh as a research assistant in the department. Dr. Singh had a very successful career at Cambridge and has published some important papers on the physiology of muscular contfactions in British Physiological Journals. He continued his researches on the Physiology of muscle in this department and also helped in pharmacological investigations on anti-malarial drugs.

The department was chiefly concerned in the investigations of properties of the two new synthetic anti-malarial drugs, viz., Plasmoquine and Atebrin. Experiments designed to study the question of filtration of plasmoquine by the placenta were completed and showed that the placenta could very efficiently filter out plasmoquine, and prevent large quantities of the drug from entering the foetal circulation. This was confirmed by actually measuring the concentrations of plasmoquine in the maternal and foetal blood simultaneously after giving a large dose of plasmoquine to the mother, the test described by us last year for estimating small quantities of plasmoquine being used for this purpose. Further it was shown that therapeutic doses of plasmoquine given during pregnancy to experimental animals did not in any way affect the litter given by these animals, the growth and general health of the young animals being in no way different from that of the controls.

The fate of plasmoquine and atebrin in the body was investigated and it was found that plasmoquine was destroyed by the liver to a considerable extent. The agent responsible for this destruction appears to be of the nature of an enzyme. Some other organs of the body also take part in destroying or converting the drug into some other substance, the brain being one of them.

The distribution of atebrin in the tissues after oral and parenteral administration of the drug was studied and it was found that the dye was especially concentrated in organs containing reticulo-endothelial cells.

Acetylcholine has now been recognized to be a chemical agent which is concerned in many a physiological phenomenon. Little however is known regarding the source of acetylcholine in the body. Investigations on this important problem were continued during the year and the acetylcholine formation by various tissues in the body was studied. The interesting fact that came to light after these investigations was that those organs which are rich in nerve cells can form acetylcholine in much larger quantities than others. This suggested that chiefly the nerve cells are concerned in formation of this chemical mediator. These findings were published in the Quarterly Journal of Experimental Physiology, London.

Investigations referred to above were made on slices of tissues which were allowed to respire in a saline medium. Further experiments were also made with the entire organs like the whole length of intestines and by employing a device which could maintain a continuous circulation of a saline medium through its vascular channels for five hours or more it was shown that acetylcholine formation can continue in such a preparation also. The amount of acetylcholine thus formed was approximately equal to 2y per gm. of the intestines of a cat.

Dr. B. K. Nandi had synthetically prepared a compound called Benzo-nicotine in Manchester. The pharmacological properties of this compound were investigated and it was found that qualitatively it had exactly the same actions as Nicotine, but quantitatively it is approximately only one-third as active as Nicotine is.

Biological Assay of Drugs.—Some samples of Tincture Digitalis manufactured in this country were assayed and one of them was found to be about twice as strong as the standard B. P. tincture. This fact again brings out the necessity of proper standardization of drugs before they are put on the market.

SECTION IV.

Department of Bio-chemistry.

This department has continued to be of increasingly great use to the other departments of the Institute and to hospitals and medical practitioners for the various bio-chemical examinations in the diagnosis of disease. The examinations carried out during the year are detailed in the table below. In addition to the routine work, research work on the study of a possible hæmoglobin constant on blood urea clearance in healthy Indians and examinations of the chemical composition of the urine of healthy Indians is being carried on. Details of researches are given in Part II of this report.

23·8 litres of horse serum have been concentrated during the year under report. The usual sodium sulphate globulin precipitation method was employed. The technique has already been described in the previous reports of this Institute (page 26, Report of the Haffkine Institute, 1932–35).

Routine Examinations carried out in 1938.

Blood—		*Urine*—	
		General Quantitative Test	97
Nonprotein Nitrogen	94	Total Nitrogen	10
Urea Nitrogen	131	Urea Nitrogen	20
Uric acid	30	Uric Acid	14
Creatinine	24	Sugar	58
Cholesterol	45	Chlorides	6
Chlorides	22	Albumin	35
Ammonia Nitrogen	3	Ammonia Nitrogen	4
Sugar	78	Total Acidity	3
Phosphorus	24	Hion Concentration	2
Serum calcium	44	Calcium	1
Acetone bodies	1	Lactose	1
Hæmoglobin	56	Phosphorus	3
Cell volume	23	Oxalic acid	1
Van den Bergh Reaction.	13	Creatinine	1
Icterus Index	1	Blood	8
Plasma Proteins	1	Bile	9
Co_2 capacity of Plasma.	2	Lead	3
	592	Urinary Calculus	2
		Diazo Test in Urine	3
			281

Cerebro-spinal Fluid—			*Miscellaneous—*		
Total Protein	..	31	Glucose tolerance test	..	11
Globulin	..	12	Kidney function test	..	37
Sugar	..	21	Liver function test	..	1
Chlorides	..	44	Gastric analysis	..	13
Nonprotein Nitrogen	..	1	Basal metabolism	..	33
Urea	..	1	Coagulation time of blood	..	4
Lange's colloidal gold Test	..	30	Formolgel test	..	3
		140	Fluids, abdominal, pleuritic, cystic, etc.	..	9
Fæces—					111
Fat in fæces	..	5			
Occult blood	..	3	Total	..	1,132
		8			

SECTION V.

Department of Entomology.

This department was organised in this Institute during the second half of the year and was put under the charge of Dr. M. Sharif, D.Sc., Ph.D., who was specially engaged for the purpose. Entomology is an important department of studies in connection with tropical diseases, and it is hoped this department will prove of great value to the Province. Important research work in connection with fleas has been started and is detailed in Part II of this report.

SECTION VI.

Department of Public Health Chemistry and Bacteriology.

Due to investigation into the origin of the outbreak of typhoid fever in Bombay City and suburbs, the work of this department was very much enhanced. Samples of water from the mains, wells, sewage from drains and various kinds of food stuffs were examined. Flies in the neighbourhood of the cases were caught and an attempt was made to isolate enteric group of organisms. Except in the case of the samples of sewage from the open-drains, Bac. typhosus could not be found in the other material examined.

The following is the résumé of the work done in the department during the year under review.

A. *Chemical Work.*

Waters.—During the year 20 samples were examined, majority of these being for sanitary purposes. The chemical composition of these showed extreme variations in total solids, chlorides, hardness, etc. as compared with the recognised chemical standards of purity.

B. *Bacteriological Work.*

The following samples were examined :—

(1) Drinking waters for standard bacteriological examination	68
(2) Drinking waters examined for typhoid ..	21
(3) Sewage and effluents for typhoid ..	14 (6 positive for B. typhosus)
(4) Swimming bath waters	16
(5) Milk samples examined for typhoid ..	41
(6) Shell fish, crabs, etc.	11
(7) Ice-cream for typhoid	1
(8) Ice-cream for bacteriological examination ..	2
(9) Aerated waters for typhoid	2
(10) Flies examined for typhoid	16
(11) Flies examined for faecal organisms ..	12

C. *Miscellaneous Work.*

Stains and standard solutions were prepared and supplied to various laboratories in this Province.

SECTION VII.

Diagnostic Department.

A. *Bacteriological Section.*

The routine work carried out is summarised in the accompanying table.

Material Examined.	Positive.	Negative.	Total.
A. Blood—			
(*a*) Films for microscopical examination—			
for malaria	4	38	42
„ relapsing fever	..	2	2
„ differential counts	..	..	80

Material Examined.	Positive.	Negative.	Total.
A. Blood—contd.			
(*b*) Counts for R.B. Cs.	..	..	53
,, ,, W. B. Cs.	..	..	47
(*c*) Cultures for organisms (B. typhosus) ..	100	335	435
(*d*) Serological examination—			
Wassermann Reaction	1,070	1,997	3,298 (215 indefinite, 16 anti-complementary.)
Khan	357	835	1,237 (45 indefinite).
Blood grouping	..	..	3
Agglutination Tests—			
for enteric group fever	385	320	705
,, undulating fever	..	6	6
,, Typhus fever	..	3	3
,, dysentery	1	..	1
B. Sputum—			
Microscopical examination for tuberculosis	8	10	18
Cultural examination for various microorganisms	5	..	5
Animals experiments for tubercle bacilli ..	..	2	2
C. Urine—			
General analysis	..	..	272
Cultural examination	77	119	196
Examination—microscopical and (or) animal experiments—			
for tuberculosis	3	5	8
,, Freidman test for pregnancy ..	2	6	8

Material Examined.	Positive.	Negative.	Total.
D. Fœces—			
(*a*) Microscopical examination for amœbæ cysts and ova.	194	278	472
(*b*) Cultural examination—			
for cholera, typhoid and dysentery ..	25	110	135
,, miscellaneous organisms ..	..	..	57
(*c*) Microscopical and animal experiments for tubercle bacilli.	..	2	2
E. Cerebro-spinal Fluid—			
(*a*) Microscopical examination for tubercle bacilli and other organisms.	6	28	34
(*b*) Cell count	..	..	58
(*c*) Cultural examination	3	24	27
(*d*) Animal experiments for tubercle bacilli.	..	2	2
F. Pus—			
(*a*) Microscopical examination for tubercle bacilli and other organisms.	5	7	12
(*b*) Cultural examination	17	9	26
(*c*) Animal experiments for tubercle bacilli.	..	4	4
G. Miscellaneous microscopical and cultural examinations—			
Smears from swabs for diphtheria ..	3	21	24
Smears for leprosy	33	6	39
,, gonococci	5	30	35
,, Leishman Donovan bodies ..	..	2	2
Smears from gastric fluid for Boas-Oppler-bacilli.	..	3	3
Smears for tubercle bacilli from pleuritic and other fluids.	2	7	9
,, Leptospira	..	2	2
Cultures from swabs for diphtheria ..	3	21	24
,, other organisms	..	..	21
Miscellaneous fluids for pathogenic organisms.	..	..	3
Foodstuffs for organisms of salomella group.	..	2	2

Material Examined.	Positive.	Negative.	Total.
Urine for gonococci	..	2	2
Catgut for sterility	..	..	1
I. Miscellaneous Animal Tests—			
For tubercle bacilli from fluids ..	..	9	9
„ diptheria	3	..	3
J. Miscellaneous Work—			
Determination of carbolic coefficients ..	..	..	1
Identification of worms	1	1	2
Identification of snakes	..	..	15
K. Supplies—			
(1) Vaccines—			
Autogenous vaccines	..	..	73
(2) Sera—			
Group sera	..	..	10 ccs.
(3) Living cultures of pathogenic organisms	..	..	2
(4) Media—			
Lœffler's serum agar tubes	..	..	717
Lowenstein medium	..	..	15
Cobra Venom solution	..	..	1,641 ampoules.
Russell's Viper Venom solution ..	..	..	151 ampoules.

B. *Pathological Section.*

During the year under review 632 pathological tissues were received for examination. The following table gives a rough classification of the nature of these tissues and their number :—

	No.	Per cent.
Non-malignant tumours	50	7·9
Malignant tumours	70	11·1
Tissues found to be normal	10	1·6
Miscellaneous pathological conditions (including research specimens).	332	52·5
Received in a state unfit for examination	3	0·5
Animal brains for Negri bodies	167	26·4
Total ..	632	....

Of the 70 specimen malignant tumours subjected to the histological examination, 45 were carcinomas, 15 sarcomas, 6 epitheliomas, 2 seminomas of the testis, 1 melanoma and 1 teratoma.

33 per cent. of epitheliomas and 62 per cent. of carcinomas occurred in the female reproductive organs, breast and cervix of the uterus being the commonest site.

Seminmas and teratoma were confined to the testis. One of the specimens examined turned out to be a lympho-sarcoma arising either from prostate or bladder. Its definite origin could not be ascertained from the history available but this case is mentioned here because lympho-sarcoma of either prostate or bladder is very rare. A boy aged 10 was admitted with symptoms of stone in the bladder. During lithola-paxy however no stone was found, but a fleshy growth was detected. At the suprapubic operation which followed the growth seemed to be an enlarged prostate and it was shelled out easily. At this time no other growth was felt in the bladder. After about 20 days the boy was discharged from the hospital as cured. This growth was not sent for examination. A fortnight later this boy was admitted again into the hospital as the scar left after the suprapubic had reopened during an accidental fall. At the exploratory laparotomy a number of soft growths, mostly sessile but a few pedunculated, were found in the bladder. These growths showed a histological picture of lympho-sarcoma.

SECTION VIII.

Anti-rabic Department.

During the year 3,045 persons applied for anti-rabic treatment at the Institute. Out of these 2,740 completed the full course of treatment, 305 were advice cases, as the biting animals which were under observation were alive and well for 10 days after having inflicted the bite and found to be not rabid.

To date three deaths from hydrophobia have been recorded at this Institute. Out of these two died during treatment and one died after treatment giving the death rate mortality to 0·1 per cent.

During the year 7,739 anti-rabic treatments were issued to the Centres. These figures include 689 treatments issued for veterinary use.

Four new Anti-rabic Centres were opened during the year bringing the total of out-centres to 87.

(1) Civil Hospital, Amracti.

(2) Mission Hospital, Jobat.

(3) State Hospital, Tonk.

(4) State Hospital, Sawantwadi.

The details of the treatments carried out at the Institute in 1937 and 1938 are summarised below :—

—	1937	1938
Number of persons applied for the treatment ..	3,498	3,045
Advice Cases	473	305
Fully treated	3,014	2,740
Percentage of Health Returns	98·8	Awaited.
Cases of Hydrophobia	3	3 (During treatment 2, after treatment 1).
Mortality rate	0·1 per cent.	0·1 per cent.
Number of Anti-rabic Centres	83	87
Number of Treatments sent to the Centres ..	6,375	7,050
Number of Treatments sent to the Veterinary Surgeons.	315	689
	6,690	7,739

Table showing the number of brains received and examined at the Institute for evidence of Rabies during the year 1938.

—	Number.	Percentage.
Received in a state unfit for examination	3	1·8
No evidence of Rabies	49	29·3
Positive for rabies (Negri bodies present)	115	68·9
Total ..	167	....

Statement showing the classification of brains and different places from where the brains were received.

——	Positive.	Negative.	Unsuitable for examination.	Total.
Received through the Bombay Veterinary College.	22	6	..	28
Received through the Bombay Police ..	2	..	..	2
Received from private owners in Bombay	4	4	..	8
Received through other places in Bombay	2	3	..	5
Received through Out-Centres ..	85	36	3	124
Total ..	115	49	3	167

Statement showing the classification of different animals with the results of their brains examinations for rabies.

——	Positive.	Negative.	Unsuitable for examination.	Total.
Dogs	108	41	3	152
Buffaloes	3	3	..	6
Jackals	2	..	..	2
Bullocks	..	2	..	2
Horse	1	1	..	2
Donkey	1	1	..	2
Deer	..	1	..	1
Total ..	115	49	3	167

SECTION IX.

INCOME AND EXPENDITURE

The following table shows the income and expenditure of the Haffkine Institute for the year 1937-38 :—

Receipts.		Expenditure.	
	Rs.		Rs.
XXVIII—Public Health—		39-D—Public Health—D—Bacteriological Laboratories, Haffkine Institute, and 39-E—Public Health—E—Pasteur Institutes—	
Sale proceeds of Sera and Vaccines, etc.	4,00,760		
		Pay of Officers ..	86,576
		Pay of Establishment ..	77,480
Miscellaneous ..	2,286	Allowances, Honoraria, etc...	12,246
		Contingencies ..	1,55,766
Total, Receipts ..	4,03,046	Total, Expenditure ..	3,32,068

PART II.

RESEARCH WORK

I. Plague Vaccine.

(SOKHEY.)

A. *Optimal Temperature of Incubation for Past. pestis Cultures.*— In a previous report (1932-35) it was reported that in the case of broth grown plague vaccine the protective power resided almost entirely in the clear supernatant fluid of the vaccine, and that the bacterial debris had little or no protective power. This observation would lead to the assumption that the ultimate protective power of a broth vaccine depends on the presence during the period of incubation of broth cultures of factors favouring the maximum growth and maximum solution of the essential part of the bacterial bodies. The factors involved would seem to be the temperature of incubation, pH of the medium, the duration of incubation and the composition of the broth. Having standardised the method of preparing broth, it was assumed that the composition of the broth remained constant for all practical purposes during the experiments to be reported below.

Experiments have been conducted to find the temperature at which maximum growth of *Past. pestis* occurs. For this purpose pH of broth was kept at 7·0, it being planned to determine the optimal pH by subsequent experiments.

Right through this work one strain of *Past. pestis* (39/B) was used. The culture was maintained on blood agar slopes and stored at 4 ± 2°C A 2mm. loopful of the growth is taken and sown into 10 c.c. of broth in a test tube of a given diameter. The growth from agar surface is well broken up while it is being sown into the broth tube. This first broth subculture is incubated for 48 hours at 27°C. From this growth 0·5 c.c. are sown into a fresh tube containing 9·5 c.c. of broth and this tube is then incubated for exactly 48 hours, at a given temperature at which the amount of growth is desired to be measured. The resultant growth is measured by colony count method.

The colony count method adopted is as follows. From the growth a series of progressive tenth dilutions are made by adding 1 c.c. of a suspension to 9 c.c. of normal saline with standardised 1 c.c. pipettes graduated to deliver. For each dilution a fresh 1 c.c. pipette is used and the fluid from the pipette is delivered sufficiently slowly to prevent droplets of fluid forming and remaining behind in the bore of the pipette. Immediately before a pipette is charged, the suspension is thoroughly shaken for not less than 3 minutes.

A dilution or dilutions are selected which will give discrete colonies on blood agar plates when incubated at 37·5°C in an atmosphere with high water vapour pressure and will yield such counts on parallel plates as will agree with the distribution of a Poisson series. Any count which

does not conform to the statistical test for this distribution is rejected. Each of the selected dilution is planted on a set of 8 rabbit blood agar Leagroux tubes. Each tube is planted with 0·05 c.c. of a dilution which is spread on about 40 sq. cm. of the surface with a special pipette.

This is a large bored 0·5 cc. pipette, 25 cm. in length and graduated into 0·05 c.c. divisions. The last 2 cm. of the tip end of the pipette is ground into a cone. This enables the pipette to be used both to deliver the inoculum and to spread it. 0·05 c.c. of a dilution was selected because it was found that it is just the right quantity to spread on 40 sq. cm. surface, without permitting any to run on to inaccessible parts of the tube. This quantity is delivered on to the middle of the agar surface and carefully spread by means of the cone end of the pipette, which is systematically passed over the area at least eight times.

The selection of a suitable dilution for planting is a matter of importance. It is found that a dilution which will give 10 to 40 colonies in each Legroux tube serves the purpose best. If the next lower dilution is used the counts tend to be lower by 10 to 30 per cent. and with the next higher dilution counts tend to be irregular. For a 48-hour growth in broth incubated at 27°C, the 10^{-6} dilution answers the requirements.

The accuracy of counts has been checked statistically. The general level of variability conforms to expectation according to Poisson distribution for small samples as suggested by Fisher (1936). The colony counts also checked up against counts under the miscroscope.

48-hour growths at different temperatures of incubation were as follows :—

Temperature of incubation	Averages of 2 to 4 counts in millions
41°C	7·3
37°C	35·7
33°C	190·0
29°C	364·0
28°C	397·0
27°C	602·2
26°C	620·5
25°C	566·5
24°C	546·6
23°C	576·0
22°C	497·0
21°C	445·5
17°C	204·0
13°C	189·0
9°C	152·0
4°C	24·9
2°C	11·8

It seems that there is a wide zone of optimal temperature of growth.

Similar determinations are being conducted to find the optimal pH for the growth of *Past. pestis.*

B. *Comparison of broth and agar vaccines.*—The work reported before showed that broth vaccines incubated at 27°C for four weeks had higher protective power than broth vaccines incubated at 37°C for the same period of time. But in the case of agar vaccines the position was reversed. Vaccines prepared from agar growths incubated for 48 hours at 37°C and made up to contain 1,500 million organisms per c.c. were found to be much more protective than similar vaccine prepared from growths incubated at 27°C for 48 hours. The number of 1,500 million organisms per c.c. of 37°C agar growth was decided by experiment so that this vaccine may have the same protective power as the broth vaccine incubated for four weeks at 27°C.

While in the case of vaccines prepared from agar growths it is easy to correctly standardise a vaccine to contain a given number of organisms, it is not possible to so standardise the broth vaccines. In the case of broth vaccine maximum growth is aimed at by prolonged incubation under suitable conditions. During the year under review variations were noticed in the broth vaccine prepared at the Institute. In view of this observation detailed studies are being made into this problem to determine whether agar growths or broth growths offer the better chance of producing vaccines of uniform protective power. The experiments conducted so far seem to show that agar growths give more readily uniform vaccines.

II. Anti-plague Serum.

(SOKHEY.)

A. *Biological Assay of Anti-plague Serum.*—On the lines of our plague vaccine assay we have worked out a method for assaying anti-plague sera. Two tests are proposed, one a prophylactic test and the other a curative test. The prophylactic test is convenient for readily comparing different sera or for measuring the state of immunisation of a horse at different stages of serum production. The curative test is designed to approximate to the actual conditions presented in the treatment of a severe case of plague, i.e., the giving of serum is commenced after bacteræmia has developed. Haffkine Institute inbred white mouse develops bacteræmia between 48 hours and 72 hours after the induction of infection with our standard infective dose given subcutaneously.

(*a*) *The Test Animal.*—Haffkine Institute inbred white mouse or any other strain of mouse equally susceptible to plague infection, i.e. the selected strain of mouse should be such that 0·25 cc. of a 10^{-7} dilution of a 48-hour growth of *Past. pestis* in nutrient broth incubated at 27°C

per animal given subcutaneously should produce a mortality of not less than 90 per cent. of the animals used—average duration of life 5 to 7·2 days. Such a dose has 6 to 12 organisms. Ten times this dose should produce uniform mortality of 100 per cent., with a lessened lease of life, 4·2 to 6 days. Mice used as test animals should be between 2½ to 4 months and should weigh between 21 to 30 g.

(*b*) *Test Infective Dose.*

1. *Selection of suitable virulent strain.*—Only such strains should be selected as produce a mortality of not less than 90 per cent. among suitable test mice with a 0·25 cc. of a 10^{-7} dilution of a 48-hour growth in nutrient broth incubated at 27° C per animal (6 to 12 organisms) given subcutaneously.

For this purpose primary cultures from severe human cases are obtained by planting venous blood on agar slopes. After four days' growth at room temperature (27°C to 32°C) they are tested for purity. After a culture is adjudged to be pure, a 2 mm. loopful is seeded in 10 cc. broth and incubated at 27°C for 48 hours. From this a second broth subculture is made by inoculating 0·5 cc. into 9·5 cc. of broth and incubating it at 27°C for exactly 48 hours. Suitable dilutions in saline are made and virulence tested. Those that answer the above test i.e. produce practically 100 per cent. mortality with 0·25 cc. of a 10^{-7} dilution of a 48-hour growth in broth incubated at 27°C, are subcultured on rabbit blood agar (5 per cent.) and stored at 4°C±2 for future use. Cultures kept this way preserve their virulence undiminished for about three years.

2. *Standard test infective dose.*—For testing the protective power of a serum ten times the minimum lethal dose of the selected strain of organism of proved high virulence is given per animal subcutaneously. This in practice is 0·25 cc. of a 10^{-6} dilution of a 48-hour growth in broth incubated at 27°C (60 to 120 organisms per mouse).

(*c*) *Measurement of the protective power of a serum.*

1. *Principle.*—The determination of the minimum amount of a given serum that will protect half the number of the mice against the standard test infective dose, given at the same time as the serum, both given subcutaneously but in different parts of the body.

2. *Method.*—For any given serum five graduated doses are decided upon, after a preliminary test, so that the (50 per cent. end point) protective dose lies about the middle of the selected series. For each dose a batch of five mice is used. Both the serum and the standard test infective dose are given at the same time subcutaneously, but in different parts of the abdominal wall.

The mice are then observed for 30 days. During this period any animals that die are post-mortemed, signs of plague are looked for and spleen smears are examined. If no plague organisms are seen heart blood

is cultured. If plague organisms are still not found the death is noted in the protocol but a note is made that it was not due to plague. Such deaths are rare. At the end of the period of observation all the mice remaining alive are killed and examined for plague.

For each test 10 mice are used as controls and they are given the standard test infective dose only. All these control animals usually die by the ninth day, and are similarly examined.

This determination is repeated to avoid odd results.

3. *Evaluation of the results.*—From the result of these two determinations 50 per cent. end point is calculated by the method of Reed and Muench (Amer. Jour. Hyg., 1938, 27, 493) or some other equally suitable statistical method.

A suitable anti-plague serum should have a minimum mouse protective dose of not more than 0·05 cc.

(*d*) *Curative Test.*

1. *Principle.*—When a mouse of a suitable strain is given the standard test infective dose subcutaneously, it develops bacteræmia within 72 hours. The idea is to find the minimum quantity of a given serum which will save a mouse when the administration of serum is commenced after the development of bacteræmia.

2. *Method.*—For any given serum five graded doses are decided upon after preliminary tests, so that the 50 per cent. end point curative dose lies about the middle of the selected series. For each dose a batch of five or six mice is used. These are first given the standard test infective dose and exactly 72 hours after are given the serum. It has been found best to give the selected doses of serum, divided up into four equal portions. The first portion is given intravenously and the remaining three portions are given subcutaneously at 24-hour intervals. Thus the serum is given on the 4th day to the 7th day of the test.

The mice are then observed for 30 days. During this period any animals that die are post-mortemed, signs of plague looked for and spleen smears examined. If plague organisms are not seen, the heart blood is cultured. If the organisms are still not found the death is noted in the protocol with the note that death was not due to plague. Such deaths are very rare. This determination is repeated to avoid odd results.

3. *Evaluation of results.*—On the results of these two determinations 50 per cent. end point is calculated by the method of Reed and Muench (Amer. Jour. Hyg., 1938, 27, 493) or some other suitable statistical method. A suitable plague serum should have a minimum mouse curative dose of 0·4 cc. or less.

B. *Field Test.*—During the period under report an opportunity occurred for testing the value of the anti-plague serum in the field at Bettiah, Bihar. At the beginning of this year a small epidemic of plague occurred at Bettiah. Bettiah Raj and Bihar Government through their Director of Public Health offered the Institute the necessary facilities for the trial. A temporary hospital was erected for the purpose and other assistance given. For all this our thanks are due to Bettiah Raj, Bihar Government and their Director of Public Health.

The trial lasted from the middle of January to the end of April. In all 139 cases were admitted to the hospital out of which 15 cases proved not to be cases of plague. Leaving out of consideration these 15 cases, there were thus 124 cases of plague.

Alternate admission was taken for treatment with serum. No selection of any kind was made. The cases not given serum, were treated with iodine solution given intravenously and were considered as controls. Of the total number of 124 cases, 69 were treated with serum. Of these 19 died giving a percentage mortality of 27 per cent. Of the 55 controls, 36 died, giving a percentage mortality of 65 per cent. The excess in the number of cases treated over those used as controls in spite of taking alternate cases, was due to the fact that during the third week of the trial all cases admitted were treated with serum for reasons over which we had no control.

The following analysis of the cases gives a better idea of the serum :—

Classification of cases.	Treated with serum.		Controls.	
	Number.	Deaths.	Number.	Deaths.
Bubonic	34	1*	16	2
Mild septicæmia (up to 40 colonies per 0·25 c.c. of blood).	15	4	15	11
Severe septicæmia (more than 40 colonies per 0·25 c.c. of blood).	20	14	24	23
Total ..	69	19	55	36

*This patient died of lobar pneumonia.

It will be noticed that the classification employed this time is somewhat different from that used on a previous occasion. It was felt that the previous classification did not represent the correct difference in the severity of different cases.

In the clinical observation of cases it was noticed that the administration of the serum markedly reduced the toxic symptoms within 24 hours : delirium, restlessness, and conjestion of the eyes disappears and temperature falls.

Comparison of these results with the results obtained with other sera.

For this purpose the figures of the first field trial given in 1936 report and of the present field trial may be added up. The sera used were practically the same.

Various sera tried in plague hospitals.	Years.	Treated.		Controls.	
		Cases.	Case mortality per cent.	Cases.	Case mortality per cent.
Yersin's	1897–1907 ..	226	74·3	231	70·5
Do.	1908–1910 ..	146	65·1	146	71·9
Lustig's	1897–1903 ..	608	71·7	609	79·1
Terni's	1901–1904 ..	110	80·9	110	81·8
Brazil's	1902–1904 ..	70	82·8	70	85·7
Rowland's	1911–1912 ..	76	68·4	76	77·6
Naidu's	1932–1933 ..	179	60·0	143	85·0
Sokhey's	1936–1937 ..	94	25·5	80	62·5*

* These controls were treated with Iodine given intravenously.

III. Chemotherapy of Plague.

(SOKHEY.)

Some of the sulphanilamide compounds which have proved of such great value in streptococcal infections were selected and experiment put up to test their curative value in plague infection. For this purpose experimental conditions were designed to approximate to condition which actual severe cases of plague present. Haffkine Institute inbred mice were used as the test animals. They were infected subcutaneously with the standard test infective dose and treatment with the selected compounds commenced 72 hours after the induction of infection when bacteræmia had developed. Preliminary experiments show that prontosil rubrum, prontosil soluble and M & B 693 have little or no curative action. While our anti-plague serum, under the same

conditions, saved practically 80 per cent. of the test animals. Experiments are in progress to test the action of these drugs under less severe conditions of infection.

IV. Serology of Plague.

A. *Precipitin Reaction.*

(WAGLE.)

Last year it was shown that horse sera contained antibodies against agar in addition to those against *Past. pestis* and consequently it became necessary to absorb the serum of its agar antibodies before determining its precipitin titre against *Past. pestis*. This method though it gave a rough idea as to the potency of the serum was found later on not to give repeatable results. The same serum, after absorption, gave widely varying results in duplicate tests and so its accuracy could not be depended upon and the method had to be abandoned.

An attempt was therefore made to get an antigen free from agar and the following method of preparation was subsequently adopted. An emulsion of *Past. pestis* in saline is prepared from an agar culture grown at room temperature for two days and this emulsion is centrifuged. The supernatant, which contains most of the agar in solution is decanted off and thrown away. The deposit of the bacilli is again suspended in saline and centrifuged. This process is continued three or four times till all the agar present in solution is removed. Finally the deposit is resuspended in saline and the suspension heated at 100° C for one hour. The supernatant obtained after centrifugalisation of this heated emulsion constitutes the antigen. This antigen reacts specifically with the anti-plague sera but the precipitin titre obtained with them is low. The results obtained with the different sera are shown below.

Horse sera.	Precipitin titre of the serum.	Protection in experimentally infected white mice.
12 ..	1 in 40 ..	Good protection.
13 ..	1 in 30 ..	Do.
14 ..	1 in 20 ..	Affords protection but protective dose is bigger than that of sera 12, 13, and 16.
15 ..	1 in 20 ..	Same degree of protection as 14 serum.
16 ..	1 in 20 ..	Good protection.

The table shows that sera Nos. 12 and 13 which give a comparatively higher titre afford good protection while sera Nos. 14 and 15 with lower

precipitin titre show less protective value. No. 16 serum is however an exception. This serum though its titre is the same as that of 14 and 15, is as potent as Nos. 12 and 13 sera. Such exceptions have been found in the different methods used so far and the matter will have to be further explored.

The optimal proportion method also has been tried using the filtrate of a broth-culture as antigen. The work is not yet complete and so definite opinion cannot be given as to its value. There are indications however that its results though giving an idea as to their potency in the case of majority of sera, would not correlate with the biological test in every case. In other words, a few sera will prove to be exceptions in this method also.

Work on both the methods described is in progress.

B. *A Precipitin reaction with a lysate of the culture of Past. pestis.*

(WATS.)

In continuing the work for a suitable serological method for the titration of anti-plague sera, it was found that the filtrate of an emulsion of *Past. pestis* to which chloroform had been added as a preservative and which had been kept in the refrigerator for a few days, gave a definite precipitin reaction with anti-plague sera. Before setting a final standard method for the preparation of this antigen an elaborate series of experiments had to be carried out. It was found that the meat digest agar growth at 37°C gave a superior antigen than the one at 27°C, the former being 6–8 times more potent than the latter.

The technique of preparation of the antigen for the test is as follows :—

Method of preparing the antigen.—Acid digest agar bottles sown with *Past. pestis* are grown at 37°C for 4 days and after this period each bottle is washed with 15 cc. of water. To this emulsion is added 1·5 cc. of $CHCl_3$, and the mixture is shaken in the shaker for one hour. This mixture is left in the refrigerator for 48 hours after which it is centrifugalised. The supernatant fluid is separated and 0·25 per cent. of carbolic acid is added as a preservative.

Another method for preparation was to make the emulsion of the growth in 0·1 per cent. Lissopol solution. Lissopol is a detergent product prepared by the Imperial Chemical Industries, Ltd. : it is a " long chain compound with the polar group SO_3 Na at its head and thus fulfils the modern conception of a substance possessing great interfacial activity ". The meat digest agar bottles are grown at 37°C for four days, washed with 0·1 per cent. aqueous solution of Lissopol previously sterilised by heating at 80° C for 20 minutes on two successive days. The emulsion is heated at 55°C for 45 minutes and left in the refrigerator for two days.

It is centrifugalised and 0·25 per cent. Phenol added as a preservative. This gives a more active antigen than the chloroform method.

Method of putting up the test.—The sera are put up for the precipitin reaction by the Dean and Webb's method of optimal proportions. Before proceeding with the accurate determination of the antigen-antibody ratio, a preliminary experiment is carried out to find out the range of the antigen dilution to be put up. Antigen dilutions ranging from 1 : 10 to 1 : 100 with a difference of 10 are added to an equal quantity of 1 : 100 dilution of serum. The mixture is incubated in a water bath at 55° C.

The results have got to be observed at very short intervals as the reaction is very rapid. Having known the point of optimal ratio the relevant and more closely spaced dilutions of the antigen are put up in the subsequent more accurate determinations.

Preliminary results.—The one unique advantage that the antigen possesses is the rapidity with which it reacts with the anti-plague sera. With a potent serum the reacting time is only about 15 minutes, and very rarely exceeds an hour.

The antigen can be prepared very easily and preserves well for a long time. One sample tested six months after the preparation did not show the slightest sign of deterioration.

The results obtained are regular and repeatable, e.g. horse sera Nos. 12, 13 and 16 have proved to be good by the biological method used at this Institute, and to give more or less similar results by this precipitin test though the order of potency is different.

It is hoped to carry out a series of parallel animal and precipitin tests on the anti-plague sera under preparation to ascertain the exact value of this method, though there is no doubt that the preliminary results are encouraging.

The following table summarises the results obtained on some of the anti-plague sera :—

Precipitin reaction with $CHCl_3$ Antigen

Serial No.	Serum particulars.	Date of collection of the serum.	Antigen antibody ratio.
1 ..	12 pooled ..	23rd March 1938 ..	8·3
2 ..	12 concentrated .	2nd March 1938 ..	12·5
3 ..	12 ..	9th March 1938 ..	8·3
4 ..	12 ..	14th April 1938 ..	7·1

Serial No.	Serum particulars.	Date of collection of the serum.	Antigen antibody ratio.
5 ..	12 concentrated	4th May 1938 ..	8·3
6 ..	13 ..	4th April 1938 ..	10·0
7 ..	13 concentrated	4th May 1938	12·5
8 ..	14 ..	10th March 1938 ..	2·5
9 ..	15 ..	10th March 1938 ..	3·6
10 ..	16 ..	11th March 1938 ..	6·25
11 ..	16 ..	30th March 1938 ..	8·3
12 ..	16 concentrated	12th April 1938 ..	6·25
13 ..	18 ..	1st April 1938 ..	5·0

C. *Study of the Antigenic Structure of Past. pestis.*

(SHRIVASTAVA.)

(1) Isolation of an active protein fraction of immunising value in the Haffkine vaccine is being made by saturating the supernatant with sodium sulphate. The precipitate is dissolved and reprecipitated with various concentrations of sodium sulphate. The products obtained are further purified, dialysed to free them from the salt. Their serological activity as well as their immunising capacity against *Past. pestis* will be tested.

(2) Recently nucleoproteins are assuming a great importance in the study of the antigenic structure of micro-organisms. So attempts are being made to isolate any nucleoprotein fraction present in the supernatant by precipitating it with dilute hydro-chloric acid in cold. This is followed by further precipitations with dilute HCl from alcoholic solutions (50 per cent.) of the precipitate. A substance has been obtained that reacts with the antiserum and gives very strong tests for protein. Further investigations are being made on the preparation and properties of this substance.

(3) Isolation of an active polysaccharide fraction from the supernatant of the Haffkine vaccine. The supernatant is concentrated in vacuo at a bath temperature of about 40° C and the concentrate is precipitated with alcohol in the presence of sodium acetate. This precipitate is further purified from protein and inorganic impurities by repeated precipitations with alcohol in the presence of acetic acid and sodium acetate, and shaking a few times with chloroform and n-Butyl alcohol. The final product gives negative test for protein and a strong Molisch test for carbohydrates. It reacts with horse as well as rabbit antisera for *Past. pestis*.

Further experiments are being made on the properties of this product, which is presumably a carbohydrate.

(4) Besides the above, a quantitative precipitin test is being developed to evaluate the potency of the antiserum obtained from horses. The supernatant of the Haffkine vaccine is being used as the antigen. The results obtained show that there is a certain degree of correlation between the precipitin content and the results of the mouse protection test, but much more work is needed to elucidate this point further.

V. The effects of temperature and humidity upon the growth of early stages of the three Indian rat-fleas.

(SHARIF.)

It is a well-known fact that the chief factors that influence the larval life of a flea are temperature, humidity and food. In order to determine the effects of different constant temperatures and relative humidities on the rate of growth of the flea larvæ the use of a standardized food whose quality and quantity could be gauged was found necessary. It was found experimentally that the dried horse blood and the medicinal yeast fulfil the ideal nutritional requirements of the rat-flea larvæ. At 27°C temperature and 80 per cent. relative humidity *Xenopsylla cheopis*, *X. astia* and *X. brasiliensis* complete their larval and pupal life within 16–23, 16–27 and 22-27 days respectively with hundred per cent. success in the rearing of these fleas. At the same temperature and 90 per cent. relative humidity the larval and pupal period for *X. cheopis* is 18 to 27 days, for *X. astia* 18 to 27 days and for *X. brasiliensis* 22 to 28 days. It appears that 27°C temperature and 80 to 90 per cent. relative humidities are within the optimum range of temperatures and relative humidities for these species. The larval and pupal life of these species is prolonged at 27°C temperature and 60 per cent. humidity, being 20–27 days for *X. cheopis*, 22–30 days for *X. astia*, and 22–30 days for *X. brasiliensis*. At this temperature and 50 per cent. humidity *X. cheopis* completes its larval and pupal life within 26 to 36 days with almost cent per cent. success in the rearing of these fleas, and *X. brasiliensis* in 33 to 37 days with about 50 per cent. mortality. On the other hand the larvæ of *X. astia* at this temperature and humidity were all killed in 1–7 days. Forty per cent. relative humidity is fatal to larvæ of all the three species. It appears that there are differential powers of resistance to desiccation in the three instances, *X. astia* being most susceptible to low humidities and *X. cheopis* the least. The larvæ of *X. cheopis* do not grow at all at temperatures 9-12°C and 80 per cent. humidity. Some of them remained alive in the first instar for more than two months. They die in a day's time at 6°C temperature and 80 per cent. humidity. At cent per cent. relative humidity and 27°C temperature the larvæ completed their larval life in 8 to 11 days but they all died in the pupal stage.

VI. On the Male Genitalia of the Indian Cat-flea.

(SHARIF.)

The homologies of the male genitalia of *Ctenocephalides felis* subsp. *orientis* (Jordan) are determined. In this species the phallus is short and cannot protrude through the external opening of the genital chamber and the two phallic apodemes carry the spermatozoa to the vagina of the female. There is a pumping sac which sucks up the spermatozoa from the genital chamber and then pumps them into the terminal tabular portion of the genital chamber from where they are carried away by the phallic apodemes.

VII. Typhoid Fever outbreak in the City of Bombay.

1. *Diagnosis of Cases.*

(WAGLE.)

In June 1938 when typhoid assumed epidemic proportions, this Institute undertook to do free laboratory diagnosis of suspected typhoid cases and investigate into the source or sources of infection. On receipt of a call from a medical practitioner, an officer of this Institute visited the patient and obtained blood sample for Widal test and blood culture. In those cases where both the Widal and blood culture proved negative, samples of fæces and urine of the patient were asked for from the relatives but actually very few samples were received for examination. Thus between 25th June to 31st December the Institute attended to 398 calls from medical practitioners for making a diagnosis of suspected cases.

Method of diagnosis—(*a*) *Blood culture.*—For these purposes, usually 10 cc. of blood was drawn asceptically from the vein of an adult patient and 5 cc. from that of a child. Half the quantity collected was then cultured into 100 cc. glucose broth and the remaining half was allowed to clot for separation of serum. The serum after separation was used for the agglutination test while the clot remaining in the tube was utilised for making a clot culture in 100 cc. of glucose broth. Cultures from both flasks were tested for growth first after two days and if negative, again after five days.

(*b*) *Widal test.*—For Widal the qualitative method of Felix was employed using the Dreyer's technique. In order to put correct interpretation on the results of the Widal, it became necessary to estimate the level of normal agglutinins in a random sample of population. As a result of this work a titre of 1 in 250 with *Bact. typhosum* "O" suspension and 1 in 125 with *Bact. typhosum* "H" suspension was taken as of definite diagnostic importance, while a titre of 1-125 with *Bact. typhosum* "O" suspension and 1-50 with *Bact. typhosum* "H" suspension was taken as suggestive of infection. A titre of 1-25 with *Bact. typhosum A.* "H" suspension was found to be diagnostic of paratyphoid "A" infection.

(c) *Culture of fæces.*—As has been mentioned earlier, very few samples of fæces were received from the practitioners for diagnostic purposes and in only two cases positive diagnosis was arrived at by fæcal culture.

For isolation of organism from fæces various media suggested for isolation of typhoid bacilli were first tried but most of them were not found to give satisfactory results. Wilson and Blair's bismuth sulphite agar as recently modified by former was found to be the best and this medium was used for isolation of organisms from the fæces of the patients as well as of suspected carriers.

Using these different methods of diagnosis, 250 cases were found to be positive out of a total of 398 cases whose samples were obtained by this Institute. 234 of these proved to be cases of typhoid fever and 16 of paratyphoid A infection. The diagnosis of 234 cases of typhoid fever was arrived at as follows. In 89 cases it was based on the isolation of *Bact. typhosum* from blood, in two cases on the isolation of causative organism from fæces, and in 143 cases on positive Widal test. Of the 16 paratyphoid A cases, 10 were diagnosed by isolation of *Bact. typhosum A* from blood, and six by positive agglutination test. It is interesting to note that no case of paratyphoid B infection was detected in these cases.

All the typhoid strains when examined serologically showed the presence of Vi-antigen, indicating a highly virulent nature of the organism causing the present epidemic.

Distribution of these 250 cases is given below :—

Ward ..	A	B	C	D	E	F	G	Suburbs, etc.
Number of cases ..	8	13	18	41	5	67	42	56

It will be seen that the cases were distributed all over the city, no ward being exempt. The wards to suffer worst, however, were F, G and D, which together contributed to 150 cases. The disease was not limited to any particular community but the cases reported were predominantly among the middle class population. It is likely that cases among the poorer sections of the population who cannot afford medical aid were not reported.

Mortality.—If mortality amongst those cases which were found to be positive bacteriologically alone is considered, it is found to work out at 10 per cent., 20 deaths being reported among 200. If, however, to these cases are added others which though bacteriologically negative are suspected to be of typhoid clinically, the mortality figure comes to 29 amongst a total of 311 cases, i.e. 9·32 per cent.

Carriers.—Since 1933 more than 300 cases have been notified to the Executive Health Officer every year and in 1937, 556 attacks were notified. With such a great incidence of disease for many years, it was expected that there would be a large number of carriers, some of

them handling milk and other articles of food and thus spreading the disease. But the examination and detection of carriers proved to be a difficult proposition. Suspected carriers often refused to give sample of their stools and the public in many cases non-co-operated in spite of the best efforts of the officers of the Department of Health. Only 88 persons could be examined for the carrier state either by a preliminary examination of blood or by the examination of fæces and urine. Usually the blood of the suspect was first examined and if blood showed a high content of H or O antibody, then samples of fæces and urine were asked for and examined. No carrier was detected amongst those examined. This investigation is, however, being continued in spite of great difficulties experienced in collection of samples.

Potency of T.A.B. Vaccine.—During the days of epidemic, attention had to be specially directed to the potency of the typhoid vaccine issued by the Institute, in order to ensure that only a potent vaccine was issued from the Institute. The potency was tested in laboratory inbred white mice by first immunising them with vaccine and subsequently giving them an infection with a highly virulent strain. It was found that the vaccine gave adequate protection against a virulent local strain as well as against the well known virulent strain Ty2.

2. *Sources and Mode of Spread.*

(WATS.)

Water.—Several samples of water from the City mains, central reservoirs, distribution pipes and domestic storage tanks were examined. Though typhoid organisms were not found in any sample, yet there was evidence that water from the Tulsi Main was below the required standard of purity for chlorinated waters, and even water from the Tansa Main was not wholly above suspicion. Water from domestic cisterns tended to be highly contaminated with fæcal organisms. Results are given in Tables I and II.

Food.—A number of samples of shell-fish and milk were examined. Infection with coliform organism of fæcal type is common, but search for *Bact. typhosum* has remained negative. In view, however, of the multiplicity of the sources of supply of milk and other foods this line of investigation is not being proceeded with further for the present. As carriers are spotted, this part of the enquiry will be taken up again.

Flies.—Eight batches of flies collected from different places in Wards F and G were examined and fæcal organisms were isolated from the body surface of the flies from seven batches. This indicates that flies were playing a part in the spread of the disease. Given basket privies and open drains into which sewage effluent, treated and untreated, flows the spread of the infection through flies can be easily imagined. Seasonal increase in the number of cases of typhoid during the months of June to October coincides with the increase in the number of flies. Wards F and G provide excellent breeding places for flies. Low lying areas between G Ward and the B. B. & C. I. Railway line are being filled

with garbage and refuse. These fillings provide excellent breeding places. I have actually seen the garbage arriving in carts at the dumping ground black with flies. Similarly a large piece of ground used for making cow-dung cakes near Haines Road, and ill-kept stables all over the City are sources of fly nuisance.

TABLE I.

Showing the results of bacteriological examination of water.

No.	Sources of samples.	Total colonies per cc. on agar.	Total colonies per cc. on bile salt agar.	Number of presumptive Coli in 100 cc and their types.	Fermentation of milk of the samples as for the Cl. Welchii Test.	Black colonies on Wilson and Blair's Sulphite Agar
1	Inlet at Malabar Hill Reservoir.	110	4	1 (Aerogenes type).	Absent in 100·0 cc.	*Nil.*
2	Inlet at Bhandarwada Reservoir.	70	*Nil.*	1 (Intermediate type).	Absent in 100·0 cc.	*Nil.*
3	Tap from Gowalia Tank Road	75	9	3 (Intermediate type).	Absent in 100·0 cc.	*Nil.*
4	Khandke Building, Block 6, 2nd Floor, Dadar.	50	5	5 (Intermediate type).	Absent in 100·0 cc.	*Nil.*
5	Bedekar Building, 1st Floor, Dadar.	31	2	3 (Intermediate type).	Absent in 100·0 cc.	*Nil.*
6	Dadar Market ..	120	10	5 (Intermediate type).	Absent in 100·0 cc.	*Nil.*
7	Tulsi water collected from Tap at Santa Cruz.	27	3	5 (Intermediate type).	Absent in 100·0 cc.	*Nil.*
8	Tansa Main, Slaughter House.	20	1	*Nil.*	Absent in 100·0 cc.	*Nil.*
9	Tansa Supply Waroda Road House.	48	1	*Nil.*	Absent in 100·0 cc.	*Nil.*
10	Tulsi Main, Nawapada Junction.	109	2	7 (Aerogenes type).	Absent in 100·0 cc.	*Nil.*
11	Tulsi Branch Sayani House.	20	*Nil.*	5 (B. Colifaecal type).	Absent in 100·0 cc.	*Nil.*

TABLE II.

Showing the results of examination of the raw water samples from the various lakes. These were collected on 12th December 1938.

No.	Sources of samples.	Total colonies per cc. on agar.	Total colonies per cc. on bile salt agar.	Number of presumptive Coli in 100 cc. and their types.	Cl. Welchii Test.	
					Fermentation of litmus milk.	Black colonies on Wilson and Blair's sulphite agar.
1	Vihar Lake	About 990	114	180 (Aerogenes group).	Absent in 100·0 cc.	Present.
2	Tulsi Lake ..	About 250	129	1,800 (Aerogenes intermediate groups).	Absent in 100·0 cc.	Present.
3	Tansa Lake ..	About 780	85	13 (Aerogenes group).	Present in 100·0 cc.	Present.

VIII. Effect of Plasmoquine and Atebrin on the Fœtus.

(DIKSHIT.)

Further experimental evidence to demonstrate the filtration of plasmoquine by the placenta was obtained by measuring the respiration of the fœtus by recording the movements of the fœtal diaphragm. The respiratory centre of the fœtus was found to be very sensitive to the action of plasmoquine even when very small quantities of the drug were introduced directly into the fœtus. When however the drug was introduced into the maternal circulation no appreciable action on the fœtal respiration could be observed. This could only be attributed to the fact that the placenta did not allow sufficient quantities of the drug to reach the fœtal circulation even though high concentrations were present in the maternal blood. Experiments were similarly made with atebrin. It was found that atebrin was also filtered out by the placenta but when sufficiently large quantities of the drug were introduced into the maternal circulation evidence of appearance of the drug could be obtained in the fœtal circulation also. The drug was injected into the mother in very high doses and concentrations which can never be reached in therapeutic use of the drug were obtained. When atebrin was given in the therapeutic doses no evidence of its appearance in the fœtal circulation was obtained.

IX. Fate of Plasmoquine in the Body.

(DIKSHIT AND NANDI.)

It has been found that when plasmoquine is injected into animals it is destroyed or converted into some other compound quickly. If for example a small dose of the drug is injected into white mice, almost 80 per cent. of it could be recovered if the mouse is killed within 15 minutes and only about 60 per cent. can be recovered if killed after two hours. Attempts were therefore made to see what organs of the body were chiefly responsible for this destruction and it appears that the liver and the brain are chiefly concerned in destroying plasmoquine. It was further observed that acetone extracts of these organs retain the plasmoquine destroying property but addition of acid to the extracts inhibit the action. The nature of this process is under investigation, but there is evidence to support the view that the agent responsible for the destruction of plasmoquine is an enzyme.

X. Concentration of Atebrin in the Blood and Tissues.

(DIKSHIT.)

It was previously observed that atebrin diffuses out very rapidly into the tissues. This diffusion of the dye was studied further and it was found that the spleen, liver and lungs were the organs into which the dye was concentrated most. After oral administration, the concentration of the drug was highest in the liver, but after intravenous injection it was found that the lungs contained almost an equally high concentration. This concentration of atebrin in organs containing reticulo-endothelial cells appears to be of significance because the reticulo-endothelial system has been suspected to be vitally concerned in malarial infections.

Estimation of Atebrin in the blood after its oral or parenteral administration revealed the remarkable fact that even after widely varying doses of the drug the concentration of atebrin in the blood came to about 1—300,000 in a short time and remained at that level for some hours. It was further observed that if atebrin was continuously infused into the vein of an experimental animal the heart continued beating till a concentration of about 1—90,000 was reached in the coronary circulation. When this limit was passed, the heart ceased to function. The diffusion of the dye in the tissues was however found to be extremely rapid and very large doses had to be given to reach the concentration of 1—90,000 in the blood.

XI. Acetylcholine Formation by Tissues.

(DIKSHIT.)

The role acetylcholine plays in mammalian physiology is now well known. Little however is known regarding the source of this substance

in the body. Experiments made last year showed that thin slices of brain and intestines when allowed to respire in a saline medium containing eserine formed measurable quantities of acetylcholine. These observations were extended to other tissues of the body and estimation of acetylcholine formed by them was made by the frog's rectus muscle preparation. It was found that plain and skeletal muscles of animals do not form any measurable quantities of acetycholine nor do the different glands of the body, except the pancreas. It was further observed that organs containing a large number of nerve cells like the brain, spinal cord or intestines formed fairly large quantities of the ester. Formation of acetylcholine by the intestines was studied in detail and after employing a number of tests it was shown that the nerve plexuses in the intestines and not the plain muscles were responsible for acetylcholine formation. A theory was thus advanced saying that acetylcholine formation was a specialized function of the nerve cells.

After completing the study of slices of tissue the whole organs are now under investigation to study their function of acetylcholine formation. For this purpose a saline medium containing eserine is circulated through the vascular channels of the organ under investigation continuously for five or more hours and the ester appearing in the perfusate assayed on the frog's rectus muscle. It has been found that the cat's intestines thus perfused can form about 2γ of acetylcholine per gm. of its moist weight.

XII. Pharmacological Action of Benzo-Nicotine.

(DIKSHIT.)

Dr. B. K. Nandi has prepared a compound synthetically from quinoline nucleus, called Benzo-nicotine. The pharmacological action of this compound was investigated and it was found that it possessed actions identical with those of nicotine. Quantitatively however it was only about $\frac{1}{3}$rd to $\frac{1}{4}$th as active as nicotine.

XIII. A Hæmoglobin Constant.

(SOKHEY AND MALANDKAR.)

It had been observed (*vide* Report of the Haffkine Institute for 1937, p. 44) that the averages obtained for the hæmoglobin content by the Van Slyke's Oxygen capacity method for normal men and women in the Bombay Presidency tallied exactly with the averages for men and women worked out in the United States of America by the same method, although the dietetic habits and the climatic conditions of the two subjects were very dissimilar. This raised a question of a possible physiological hæmoglobin constant. A study for its investigation was begun, and eight cases were examined. The study has been continued during the year under report and eleven more cases have been examined.

Brilliant vital red was used instead of congo red for determining the total volume of circulating blood. Three cases out of the eleven examined have a relationship of 349·0 g. to 351·4 g. haemoglobin per square metre of body surface, while for five cases the range is 327·5 g. to 351·4 g. and for ten cases the range is 291·7 g. to 351·4 g. haemoglobin per square metre of body surface.

XIV. Study of Blood Urea Clearance in normal Subjects.

(GOKHALE.)

Moller, McIntosh and Van Slyke (Jour. Clin. Invest. 1929, 6,427) have shown that to determine the urea excreting ability of the kidney a comparison must be made of the blood urea content and the urea elimination in the urine and that the best means available to express the relation between the two is by the blood urea clearance which represents the number of cubic centimetres of blood completely cleared of urea by renal excretion. They have established a relation between the urea in the urine (U), the volume of urine per minute (V), and the urea in blood (B) in a formula and determined the mean value of the clearance for an average adult.

It has been found here, that in the case of normal Indians while their blood urea content is within the same limits as that of American normal subjects, the urinary urea content, on the other hand, is about half of American subjects. In view of this fact, it was thought worth while to see whether the formula given by Moller, McIntosh and Van Slyke will hold good in the case of Indians. A study has been started to determine blood urea clearance values for normal Indian subjects a few subjects have been studied in the year under report.

XV. A comparative study of the effect of the interaction of Ions, drugs and electrical stimulation as indicated by the contraction of unstriated muscle.

(INDERJIT SINGH.)

The experiments performed on *Mytilus* unstriated muscle, at the Physiological Laboratory, Cambridge, have been extended to the unstriated muscle of the frog stomach. The environment of *Mytilus* muscle is quite different from that of frog stomach, and it was deemed expedient to repeat some of the experiments performed on *Mytilus* muscle, on the plain muscle of frog stomach, in order to see whether some of the conclusions arrived at previously were of general applicability.

It was found that many of the differences between the responses of *Mytilus* and frog muscles could be accounted for by the difference in the composition of their media. The sodium, calcium and potassium

contents of sea water are respectively higher than those of the frog saline. The high calcium content of sea water renders the *Mytilus* muscle capable of withstanding high concentrations of abnormal substances. The high sodium chloride content of sea water imparts greater tonicity to contractions of *Mytilus* muscle ; this high sodium chloride content of their media is probably responsible for the high viscosity exhibited by the muscles of these molluscs.

The excitability of plain muscle appears to be controlled by at least two factors. One of these factors is stabilised by calcium, and is probably the surface membrane. The second factor is probably the difference in the concentration of ions within and without the muscle fibres. Substances affect the excitability of plain muscle and directly and indirectly ; the latter by affecting the tone. Any change in the tone is accompanied by an opposite change in the excitability to alternating current. Plain muscle exhibits two kinds of tone ; one is antagonistic to alternating current, and the other not. One kind of tone is probably accompanied by increase in viscosity to enable plain muscle to maintain contraction without expenditure of energy ; the other is akin to tetanus caused by alternating current or nervous stimulation.

Osmotic pressure changes the concentration of ions within the muscle fibres. The effects of electrical stimulation resemble those of increase of osmotic pressure and of calcium, and so it is concluded (1) that during electrical stimulation the concentration of ions in the fibres increases, probably by mobilisation of potassium, so that the latter moves into the muscle membrance, (2) that calcium is released. This release of calcium is probably responsible for adaptation to electrical as well as chemical stimulation, calcium being one of the factors that affects the two excitabilities similarly.

XVI. Some experimental observations on human and rat leprosy and their significance in the pathogenesis and treatment of the disease.

(Row.)

The observations recorded in this note are based on a serological study of a large number of human leprosy sera and on the histological findings of lesions found in experimental rat leprosy.

The serological results confirm the author's observations published in 1923 that with autolysate product of human tubercle bacillary culture, it is possible to demonstrate antibodies, by complement fixation, in well marked nodular leprosy sera : incidentally the findings have revealed the interesting fact in support of the general clinical classification now adopted by leprologists into three main divisions, viz. cutaneous, nerve and tuberculoid types of the disease. The analysis of over one hundred

leprous sera tested for complement fixation brings out the following facts—

(*a*) In cases of cutaneous leprosy with massive acid-fast bacillary invasion of the skin one obtains a strong complement fixation reaction in 100 per cent.

(*b*) In the nerve type of leprosy where no bacilli are demonstrable in the skin one gets a negative complement fixation in 100 per cent., as is also the case with non-leprous sera.

(*c*) In those cases known as tuberculoid, where the skin lesion is characterized by cell infiltration and thickening associated with few bacilli, the reaction gives a doubtful and indefinite result.

In discussing these results, the possibility of some other etiological factor than B leprae alone in the disease, is touched upon and a suggestion is made as to possibility of the disease, viz. of the nature of neoplasm formation where primarily localised cell proliferation in the nerves is the chief feature and it is only after the dissemination of these cells by metastasis into the skin that the acid-fast bacilli assert themselves and proliferate abundantly with the simultaneous evidence of strong positive complement fixation in the sera. Histological studies of early and late lesions in rat leprosy seem to confirm the view suggested.

A brief reference is made as to the practical applicability of the antigen in those types of leprosy where the antibody formation is found to be nil or feeble by a negative or poor complement fixation result. The following conclusions were reached :

(1) The clear-cut results obtained in the complement fixation test give strong support to the clinical division of leprosy into three types.

(2) The positive findings in all cases of cutaneous leprosy abounding in acid-fast bacilli, the negative results, obtained in all cases of nerve leprosy and the intermediate findings in the tuberculoid type of leprosy indicate that the immunity response is provoked by the acid-fast bacilli in the skin. On this was based the rationale of the therapeutic application of the tubercular autolysate-antigen in leprosy.

(3) Incidentally the results raise the question as to whether B leprae is the only etiological factor concerned in the pathogenis of the disease.

XVII. The study of the Metabolism of the Plague Bacillus.

(Sadashiva Rao.)

There is little exact and systematic information on the metabolism of the plague bacillus. An effort has therefore been made to obtain a general idea of the more fundamental aspects of its metabolism, as well as to investigate in detail those lines of work which promise to yield results of special interest. The first question taken up is the nutrition of the plague bacillus, regarding which some results were described in the previous report.

Nutritional Requirements.—The nutritional requirements of the plague bacillus, which have been investigated under conditions rigorously excluding the presence of all compounds except those added from outside, reveal two peculiarities. Firstly both the virulent strains as well as the avirulent strains derived from them by repeated culturing, have identical nutritional requirements; and secondly, accessory growth factors or "bacterial vitamins" are not required. Three amino acids only, proline, phenylalanine and cystine are indispensable and must be present in the medium before growth can occur, while the omission of glycine in general delays growth. The most important practical application of these facts is in connection with the designing of new media, with properties varying according to different experimental needs, when the provision of the three amino acids only ensures the completeness or the sufficiency of the medium to support the growth of the bacillus. For example, a medium which is protein-free is considerably superior to the usual laboratory media for the isolation in pure form proteins and polysaccharides from bacterial cultures. For such work on the plague bacillus an inexpensive protein-free medium is provided by a hydrolysate of gelatin to which cystine has been added. With a concentration of 2·3 mg. total nitrogen per c.c. this medium produces a growth of the plague bacillus which is as luxuriant as that produced by any of the usual liquid media.

Oxidation Reactions.—The next problem taken up is the study of the oxidative abilities of the plague bacillus. Using standardised washed suspensions of the bacilli, the oxidation of series of fifty different substrates including carbohydrates, organic acids and amino acids has been studied with the assistance of the Warburg manometric technique. The rate of oxidation of each substrate has been determined, after correcting for controls as cu.mm. oxygen utilised by 1 mg. bacteria (dry weight) in one hour at 0·05 M. concentration of the substrate, the oxidation being conducted in air at 27°C. These results have brought out several points of special interest. Among the sugars, glucose, fructose, mannose and galactose are oxidised at a high rate. Arabinose is oxidised at a higher rate than xylose, while rhamnose is intermediate. Of the oligosaccharides, maltose is oxidised at a high rate, while both lactose and sucrose are oxidised to a slight extent, the former more than the latter. Raffinose, contrary to the current opinion, is oxidised. Of the sugar alcohols mannitol is oxidised at a moderate rate, dulcitol slightly while sorbitol is not oxidised. Glycerol and ethyl alcohol are totally unattacked. All the lower members of the monobasic fatty acids tested were not oxidised, with the sole exception of acetic acid. The action of the bacillus on amino acids is also highly selective. Thus while alanine, proline, phenylalanin, cystine, cystein serine and glutamic acid are oxidised, the following are left untouched; leucine, isoleucin, valine and aspartic acid. Glycine is oxidised at a very slow rate. The detailed results of these experiments are being published elsewhere. Further work on the path of carbohydrate breakdown and the role of respiratory catalysts and related questions is being actively pursued.

XVIII. A new group of bacilli associated with sprue and pernicious anæmia.

(GORE.)

A new group of bacilli has been found to be associated with sprue and pernicious anæmia. These bacilli are strongly hæmolytic and capable of producing hydrogen sulphide in their litmus milk cultures. It is by means of these two characters that these bacilli are distinguished from the colon bacilli which normally occur in the intestines and typhoid or dysentry bacilli.

It is interesting to note that the bacilli of the new group are found to be common in water and sewage, and are more resistant to chlorination than the colon bacilli, on the numerical occurrence of which latter standardization of purified water is based. A new test has therefore been suggested for use as supplementary to the test in routine use in the bacteriological examination of water. In view of the general belief that sprue and pernicious anæmia are caused by dietary deficiency it is here suggested that the changes produced by the deficiency in the gastro-intestinal tract allow the bacilli of the new group to survive in the tract which under other conditions are killed in the tract.

The association of the new bacilli with sprue and pernicious anæmia has been used as an aid in the diagnosis and prognosis of these two diseases. Whether these bacilli play any part in the symptomatology of these two diseases is a question which awaits investigation.

LIST OF PAPERS PUBLISHED FROM THE HAFFKINE INSTITUTE DURING THE YEAR 1938.

1. *Dikshit, B. B.*—" Pharmacology of plasmoquine with special reference to its action ".—Procd. Nat. Inst. Science, Vol. IV, No. 2, 1938, pp. 161-164.

2. *Dikshit, B. B.*—" Acetylcholine formation by tissues ".—Quart. Jour. Experimental Physiol., Vol. 28, No. 3, 1938, pp. 243–251.

3. *Gokhale, S. K.*—" Non-protein nitrogenous constituents of blood ". A study based on the examination of 126 normal Indian men.—Sent for publication to the Ind. Jour. of Medical Research.

4. *Mahal, H. S.*—" Studies on blood choline esterace ".—Ind. Jour. of Medical Research, Vol. 25, No. 3, January 1938, pp. 703–712.

5. *Nandi, B. K.* and *Dikshit, B. B.*—" A new colorimetric test for plasmoquine ".—Ind. Jour. of Medical Research, Vol. 25, No. 4, April 1938, pp. 937–950.

6. *Row, R.*—" Some experimental observations on Human and Rat Leprosy and their significance in the pathogenesis and treatment of the disease ".—In press.

7. *Sharif, M.*—"Medical and veterinary importance of fleas and ticks and the possibilities of their control".—Current Science, VII, 1938, pp. 11–15.

8. *Sharif, M.*—"A proposal to advance the teaching and research in entomology in India".—Jour. Bom. Nat. Hist. Soc., XL, 1938, pp. 508–512.

9. *Sharif, M.*—"Diseases transmitted by the Indian species of ticks and possibility of their prevention through biological control".—"Ind. Jour. Vet. Sci., Anim. Husb., VIII, 1938, pp. 353–366.

10. *Singh, Inderjit.*—"A comparative study of the effect of the interaction of ions, drugs and electrical stimulation as indicated by the contraction of unstriated muscle. I. The effect of certain drugs and ions on the response to electric current, potassium and acetylcholine".—In press.

11. *Singh, Inderjit.*—"A comparative study of the effect of the interaction of ions, drugs and electrical stimulation as indicated by the contraction of unstriated muscle. II. The effect of sodium chloride and osmotic pressure on excitability".—In press.

12. *Singh, Inderjit.*—"A comparative study of the effect of the interaction of ions, drugs and electrical stimulation as indicated by the contraction of unstriated muscle. III. The effect of stimulation on excitability".—In press.

13. *Sokhey, S. S., Gokhale, S. K., Malandkar, M. A. and Bilimoria, H. S.*—"Red cell, hæmoglobin, colour index, saturation index, and volume index standards, Part II, normal Indian women. A study based on the examination of 101 women.—Indian Jour. of Med. Research, 25, 723, 1938.

14. *Sokhey, S. S. and Malandkar, M. A.*—"Basal metabolism of Indians".—In press.

15. *Wats, R. C. and Bharucha, K. H.*—"Larvicides for anti-mosquito work with special reference to cashew-nut shell oil.—Jour. Malaria Inst. Ind., Vol. 1, No. 2, June 1938, pp. 217–219.

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PART III.

APPENDIX.

Statistics of Anti-Rabic Inoculation.

(Standard Tables approved for use in India.)

1937.

APPENDICES.

TABLE I.

A General Statement regarding Patients treated at the Institute and its Centres from 1922 to 1937.

Year.		Europeans.				Asiatics.				Europeans and Asiatics combined.			
		Total treated.	Total deaths.	Total death rate per cent.	Advice cases.	Total treated.	Total deaths.	Total death rate per cent.	Advice cases.	Total treated.	Total deaths.	Total death rate per cent.	Advice cases.
1922...	Institute ...	64	.....	.....	.....	365	4	1·04	175	429	4	0·89	175
	Centres ...	7	.....	.....	.....	236	.....	.....	.....	243	.....	.....	.....
	Combined ...	71	.....	.....	.....	601	4	0·66	175	672	4	0·61	175
1923...	Institute ...	115	.....	.....	59	730	10	1·37	359	845	10	1·18	418
	Centres ...	38	.....	.....	.....	1,521	12	0·78	260	1,559	12	0·76	260
	Combined ...	153	.....	.....	59	2,251	22	0·97	619	2,404	22	0·91	678
1924...	Institute ...	62	1	1·61	41	525	9	1·71	372	587	10	1·70	413
	Centres ...	85	.....	.....	.....	2,880	28	0·97	330	2,965	28	0·94	330
	Combined ...	147	1	0·6	41	3,405	37	1·08	702	3,552	38	1·06	743
1925...	Institute ..	42	.....	.....	27	583	6	1·02	219	625	6	0·96	246
	Centres ...	187	.....	.....	5	3,569	28	0·78	393	3,756	28	0·78	398
	Combined ...	229	.....	.....	32	4,152	34	0·81	612	4,381	34	0·79	644
1926...	Institute ...	60	.....	.....	51	588	5	0·87	440	648	5	0·76	491
	Centres ...	287	.....	.....	20	3,861	25	0·64	322	4,148	25	0·60	342
	Combined ...	347	.....	.....	71	4,449	30	0·67	762	4,796	30	0·60	833
1927...	Institute ...	51	.....	.....	37	678	6	0·88	468	729	6	0·82	505
	Centres ...	303	.....	.....	31	3,857	25	0·64	490	4,160	25	0·62	521
	Combined ...	354	.....	.....	68	4,535	31	0·68	958	4,889	31	0·61	1,026
1928...	Institute ...	39	.....	.....	40	679	6	0·88	418	718	6	0·83	458
	Centres ...	216	.....	.....	27	4,166	34	0·81	467	4,382	34	0·77	494
	Combined ...	255	.....	.....	67	4,845	40	0·82	885	5,100	40	0·78	952
1929...	Institute ...	35	.....	.....	43	650	5	0·77	522	685	5	0·73	565
	Centres ...	286	.....	.....	19	4,621	43	0·93	507	4,907	43	0·89	526
	Combined ...	321	.....	.....	62	5,271	48	0·91	1,029	5,592	48	0·89	1,091
1930...	Institute ...	26	.....	.....	34	710	.....	.....	401	736	.....	.....	435
	Centres ...	314	1	0·29	10	4,692	50	1·07	92	5,006	51	1·01	102
	Combined ...	340	1	0·29	44	5,402	50	1·093	493	5,742	51	0·89	537

1931...	Institute	...	41	.....	.....	42	1,020	3	0·29	394	1,061	3	0·28	436
	Centres	...	326	.....	.....	3	5,670	40	0·70	80	5,998	40	0·69	83
	Combined	...	360	.....	.....	45	6,690	43	0·64	474	7,059	43	0·60	519
1932...	Institute	...	40	.....	.....	52	1,207	3	0·24	457	1,247	3	0·24	509
	Centres	...	252	.....	.....	8	5,984	60	1·00	35	6,236	60	0·96	43
	Combined	...	292	.....	.....	60	7,191	63	0·87	492	7,483	63	0·84	552
1933...	Institute	...	59	.....	.....	62	1,535	2	0·13	722	1,594	2	0·12	784
	Centres	...	298	.....	.....	8	6,139	31	0·50	32	6,437	31	0·48	40
	Combined	...	357	.....	.....	70	7,674	33	0·43	754	8,031	33	0·41	824
1934...	Institute	...	45	.....	.....	49	1,785	4	0·22	665	1,830	4	0·21	714
	Centres	...	344	.....	.....	24	5,462	23	0·42	21	5,806	23	0·39	45
	Combined	...	389	.....	.....	73	7,247	27	0·37	686	7,636	27	0·35	759
1935...	Institute	...	70	.....	.....	64	2,202	4	0·18	593	2,272	4	0·17	657
	Centres	...	306	.....	.....	7	5,544	32	0·57	44	5,850	32	0·54	51
	Combined	...	376	.....	.....	71	7,746	36	0·46	637	8,122	36	0·44	708
1936...	Institute	...	81	.....	.....	55	2,250	2	0·08	567	2,331	2	0·08	622
	Centres	...	246	.....	.....	10	5,515	29	0·52	28	5,761	29	0·50	38
	Combined	...	327	.....	.....	65	7,765	31	0·39	595	8,092	31	0·38	660
1937...	Institute	...	121	.....	.....	53	2,892	3	0·10	420	3,013	3	0·1	473
	Centres	...	255	.....	.....	8	6,590	45	0·6	34	6,845	45	0·6	42
	Combined	...	376	.....	.....	61	9,482	48	0·5	454	9,858	48	0·4	515
Grand Total		...	4,703	2	0·04	889	88,706	577	0·6	10,327	93,409	579	0·6	11,216

TABLE II.

A General classification of Patients attending the Institute during 1937.

Institute.

	Europeans.					Asiatics.				
	Class of case treated.					Class of case treated.				
	I	II	III	IV	Total.	I	II	III	IV	Total.
Number fully treated	80	15	26	...	121	67	143	2,571	111	2,892
Percentage to total	66·1	12·4	21·5	...	...	2·2	4·8	89·1	3·9	...
Total deaths	...	...	...	...	...	...	...	...	3	3
Total death rate per cent.	...	...	...	...	...	...	...	...	2·7	0·10
Percentage of health returns					100%					97·8%
Number not fully treated (presumably at risk)					...					11
Advice cases (not at risk)					...					420
Cases excluded from the statistical tables for other reasons					53					...
Total ...					53					431
Grand Total ...					174					3,323

TABLE II (*a*).

A general classification of patients attending the Centres during 1937.

Numerators = Deaths. *Denominators* = Cases

	Europeans.					Asiatics.				
	Class of case treated.					Class of case treated.				
	I	II	III	IV	Total.	I	II	III	IV	Total.
Number fully treated	175	32	31	17	255	252	922	4,033	1,383	6,590
Percentage to total	68·8	12·5	12·1	6·6	...	3·8	13·9	62·2	20·1	...
Total deaths	...	...	...	...	...	...	2	19	24	45
Total death rate per cent.	...	...	...	...	...	...	0·2	0·4	1·7	0·6
Percentage of health returns					92·3					86·8
Number not fully treated (presumably at risk)					3					1,205
Advice cases (not at risk)					8					34
Cases excluded from the statistical tables for other reasons					...					...
Total ...					11					1,239
Grand Total ...					266					7,829

TABLE III.

Classification of patients according to position of wounds, interposition of clothing and probability of rabies in the biting animal.

Numerators = Deaths. *Denominators* = Cases.

Institute.

A—Animal proved rabid.
B—Animal certified rabid.
C—Animal probably rabid according to history.
D—History of animal unknown.
E—Animal probably not rabid according to history.

	Europeans.							Asiatics.							Total	Death rate per cent.
	A	B	C	D	E	Total.	Death rate per cent.	A	B	C	D	E	Total.	Death rate per cent.		
Bitten on face	...	...	...	...	...	...	...	...	2	1/11	1/74	...	2/87	2·3	2/87	2·3
Bitten on trunk and extremities (bare skin).	...	...	24	8	...	32	...	...	12	1/512	1,538	...	1/2,062	0·04	1/2,094	0·04
Bitten on trunk and extremities (through clothing).	...	...	8	1	...	9	...	...	64	102	509	...	675	...	684	...
"Licks" on any part of the body.	65	14	...	1	...	80	...	52	9	6	1	...	68	...	148	...
Total ...	65	14	32	10	...	121	...	52	87	2/631	1/2,122	...	3/2,892	0·01	3/3,013	0·1
Percentage to total ...	53·7	11·6	26·4	8·3	...	4·2	...	1·8	3·0	21·8	73·4	...	96·8	...	...	...
Death rate ...	...	...	...	...	...	...	...	...	...	0·8	0·04	...	0·1	...	0·1	...

TABLE III (*a*).

Classification of patients according to position of wounds, interposition of clothing and probability of rabies in the biting animal.

Numerators = Deaths. *Denominators* = Cases.

Asiatic.
Centres.

A—Animal proved rabid.
B—Animal certified rabid.
C—Animal probably rabid according to history.
D—History of animal unknown.
E—Animal probably not rabid according to history.

—	Europeans.							Asiatics.							Total.	Death rate per cent.
	A	B	C	D	E	Total.	Death rate per cent.	A	B	C	D	E	Total.	Death rate per cent.		
Bitten on face	...	...	1	1	...	2	...	8	1/12	12/298	1/44	...	14/362	3·8	14/364	3·8
Bitten on trunk and extremities (bare skin).	2	6	30	16	...	54	...	117	2/59	23/3,219	2/724	...	27/4,119	0·6	27/4,173	0·6
Bitten on trunk and extremities (through clothing).	...	..	18	5	...	23	...	...	...	3/1,075	1/791	...	4/1,866	0·2	4/1,889	0·2
"Licks" on any part of the body.	35	67	55	19	...	176	...	41	66	115	21	...	243	...	419	...
Total ...	37	73	104	41	...	255	...	166	3/137	38/4,707	4/1,580	...	45/6,590	0·6	45/6,845	0·6
Percentage to total ...	14·5	28·6	40·8	16·1	...	4·8	...	2·5	2·1	71·5	23·9	...	95·2	...	...	...
Death rate ...	...	...	...	...	...	...	...	...	2·1	0·8	0·2	...	0·6	...	0·6	...

TABLE IV.

Age and sex distribution of patients treated at the Institute.

Numerator = Deaths. Institute. *Denominators* = Cases.

Age in years.	Europeans.				Asiatics.				Europeans and Asiatics combined.	
	Males.	Females.	Total.	Death rate per cent.	Males.	Females.	Total.	Death rate per cent.	Total.	Death rate per cent.
0—4 ...	7	2	9	.....	2/54	31	2/85	2·3	2/94	2·1
5—9 ...	7	3	10	.....	1/219	92	1/311	0·3	1/321	0·3
10—14 ...	6	5	11	.....	292	62	354	.....	365	.....
15—19 ...	3	4	7	.....	298	44	342	.....	349	.....
20—29 ...	18	12	30	.....	638	81	719	.....	749	.....
30—39 ...	13	14	27	.....	485	85	570	.....	597	.....
40—49 ...	9	7	16	.....	224	63	287	.....	303	.....
50—59 ...	6	5	11	.....	102	36	138	.....	149	.....
60—69 ...	.....	.....	.....	.....	37	36	73	.....	73	.....
70 and over ...	.....	.....	.....	.....	5	8	13	.....	13	.....
Total ...	69	52	121	.....	3/2,354	538	3/2,892	0·1	3/3,013	0·1

TABLE IV (*a*).

Age and sex distribution of patients treated at the Centres.

Numerator = Deaths. *Denominators* = Cases.

Centres.

Age in years.	Europeans.				Asiatics.				Europeans and Asiatics combined.	
	Males.	Females.	Total.	Death rate per cent.	Males.	Females.	Total.	Death rate per cent.	Total.	Death rate per cent.
0—4 ...	14	6	20	.....	1/148	1/99	2/247	0·8	2/267	0·7
5—9 ...	9	5	14	.....	8/727	4/195	12/922	1·3	12/936	1·2
10—14 ...	13	11	24	.....	8/665	2/191	10/856	1·1	10/880	1·1
15—19 ...	6	14	20	.....	359	98	457	.....	477	.....
20—29 ...	73	18	91	.....	6/1565	374	6/1939	0·3	6/2,030	0·2
30—39 ...	33	19	52	.....	2/913	3/254	5/1,167	0·4	5/1,219	0·4
40—49 ...	14	15	29	.....	2/437	1/190	3/627	0·4	3/656	0·4
50—59 ...	2	2	4	.....	146	2/101	2/247	0·8	2/251	0·8
60—69 ...	.....	.....	.....	.....	3/61	2/25	5/86	5·8	5/86	5·8
70 and over ...	1	.....	1	.....	29	13	42	.....	43	.....
Total ...	165	90	255	.....	30/5,050	15/1,540	45/65,90	0·6	45/6,845	0·6

TABLE V.

Classification according to number and position of wounds.

Numerators = Deaths. *Denominators* = Cases.

Europeans.

Institute.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg	...	4	6	1	2	2	...	...	...	...	...	...	...	...	...	...	15	...
Trunk	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Arm	80	9	8	2	3	1	...	...	...	1	...	...	...	...	...	...	104	...
Multiple ...	...	...	...	...	...	1	...	...	...	...	1	...	...	...	...	...	2	...
Face	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Multiple face ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total ...	80	13	14	3	5	4	...	...	...	1	1	...	...	...	...	...	121	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Separate table for Europeans and Asiatics : Also for Institute and Centres :—

(V) Europeans—Institute.
(V-a) „ —Centres.
(V-b) Asiatics —Institute.
(V-c) „ —Centres.

TABLE V-a.

Classification according to number and position of wounds.

Numerators = Deaths. *Denominators* = Cases.

Europeans.
Centres.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg	...	12	10	6	5	2	1	...	2	...	...	...	...	...	...	1	39	...
Trunk	...	...	2	1	...	...	1	...	...	...	...	...	...	...	...	...	4	...
Arm	176	17	9	4	1	2	...	1	...	...	...	...	...	...	...	...	210	...
Multiple ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Face	...	...	1	1	...	...	...	...	...	...	...	...	...	...	...	...	2	...
Multiple face ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total ...	176	29	22	12	6	4	2	1	2	...	...	...	...	...	...	1	255	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Separate table for Europeans and Asiatics : Also for Institute and Centres :—

(V) Europeans—Institute.
(V-a) ,, —Centres.
(V-b) Asiatics —Institute.
(V-c) ,, —Centres.

TABLE V-b.

Classification according to number and position of wounds.

Numerators = Deaths. *Denominators* = Cases.

Asiatics.

Institute.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg ...	...	321	736	332	338	99	64	1/38	11	7	3	4	1	...	1	1	1/1956	0·05
Trunk ...	...	11	16	6	7	2	...	2	...	...	...	...	...	...	...	...	44	.....
Arm ...	68	124	226	113	97	46	28	17	10	2	5	1	...	...	4	1	742	.....
Multiple ...	...	1	3	4	10	6	9	12	5	4	4	5	...	...	...	...	63	.....
Face ...	...	19	21	8	16	5	2	...	1	...	...	1/1	...	...	...	...	1/73	1·3
Multiple face ...	...	...	...	2	4	2	...	1	...	...	...	1	...	1	1/1	2	1/14	7·1
Total ...	68	476	1,002	465	472	160	103	1/70	27	13	12	1/12	1	1	1/6	4	3/2892	0·1
Percentage death rate. ...	...	...	...	...	...	...	...	1·4	...	...	...	8·3	...	...	16·6	...	0·1	.....

Separate table for Europeans and Asiatics ; Also for Institute and Centres :—

(V) Europeans—Institute.
(V-a) ,, —Centres.
(V-b) Asiatics—Institute.
(V-c) ,, —Centres.

TABLE V-c.

Classification according to number and position of wounds.

Numerators = Deaths. *Denominators* = Cases.

Asiatics.

Centres.

Position.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
Leg ...	...	609	3/1205	3/982	4/629	2/203	145	33	42	10	13	7	7	2	1	1/2	13/3890	0·3
Trunk ...	...	33	1/71	1/80	44	10	7	2	2	1	...	...	...	...	...	...	2/250	0·8
Arm ...	243	1/279	2/457	2/330	4/249	2/97	52	26	24	13	4	4	1/4	1	2	4	12/1789	0·6
Multiple ...	...	...	27	45	1/64	33	1/45	22	1/21	8	11	6	5	2	4	1/6	4/299	1·3
Face ...	...	3/49	2/78	1/55	2/50	1/5	4	3	1	...	...	...	...	1/1	...	...	10/246	4·0
Multiple face ...	...	...	10	1/10	18	6	2/26	12	1/8	3	4	4	6	2	2	5	4/116	3·4
Total ...	243	4/970	8/1848	8/1502	11/1054	5/354	3/279	98	2/98	35	32	21	1/22	1/8	9	2/17	45/6530	0·6
Percentage death rate.	...	0·4	0·4	0·5	1·04	1·4	1·0	...	2·0	...	...	...	4·5	12·5	...	11·7	0·6	...

Separate table for Europeans and Asiatics : Also for Institute and Centres.

(V) Europeans—Institute.
(Va) „ —Centres.
(Vb) Asiatics—Institute.
(Vc) „ —Centres.

TABLE VI.

Classification according to number and depth of wounds and interposition of clothing.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Institute.

Deep Bare.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.	Total.	Percentage death rate.
+ +	...	5	2	...	3	1	...	...	...	1	1	...	...	...	...	...	13	...
+ −	...	...	2	1	1	1	...	...	...	...	...	...	...	...	...	...	5	...
− +	80	7	7	2	1	2	...	...	...	...	...	...	...	...	...	...	99	...
− −	...	1	3	...	...	...	...	...	..	...	...	...	...	...	...	...	4	...
Total ...	80	13	14	3	5	4	...	...	...	1	1	...	...	...	...	...	121	...
Percentage death rate.	...	...	...	...	...	...	....	...	...	...	...	...	...	..	...	...	...	...

TABLE VI-a.

Classification according to number and depth of wounds and interposition of clothing.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Centres.

Deep Bare.	Number of wounds.																Total.	Percentage death rate
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
+ +	...	6	13	2	2	...	...	...	1	...	...	...	...	...	...	...	24	...
+ —	...	4	3	1	...	1	1	1	1	...	...	...	...	...	...	...	12	...
— +	176	16	5	6	3	1	1	...	...	...	...	...	...	...	...	...	208	...
— —	...	3	1	3	1	2	...	...	...	...	...	...	...	...	...	1	11	...
Total ...	176	29	22	12	6	4	2	1	2	...	...	...	...	...	...	1	255	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VI-b.

Classification according to number and depth of wounds and interposition of clothing.

Numerators = Deaths. Asiatics. *Denominators* = Cases.

Institute.

Deep Bare.	Number of wounds.																Total	Percentage death rate
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
+ +	...	333	784	286	273	39	50	1/25	8	3	1	1/5	...	1	1/4	2	3/1814	0·16
+ —	...	93	117	110	125	64	17	14	10	4	1	1	1	...	...	1	558	...
— +	68	27	81	47	56	44	27	26	7	4	8	5	...	...	2	1	403	...
— —	...	23	20	22	18	13	9	5	2	2	2	1	...	...	...	...	117	...
Total ...	68	476	1002	465	472	160	103	1/70	27	13	12	1/12	1	1	1/6	4	3/2892	0·1
Percentage death rate.	...	...	...	...	...	...	...	1·4	...	...	...	8·3	...	...	16·6	...	0·1	...

TABLE VI-c.

Classification according to number and depth of wounds and interposition of clothing.

Numerators = Deaths. Asiatics. *Denominators* = Cases.

Centres.

Deep Bare.	Number of wounds: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over	Total	Percentage death rate.
+ +	...	3/551	6/794	7/1056	8/700	5/204	3/193	66	2/63	26	23	16	15	1	4	4	2/7	37/3732	0·9
+ —	...	1/173	402	211	2/191	92	43	16	22	4	5	3	1/4	2	3	5	4/1176	0·3
— +	243	118	1/372	1/107	1/77	26	22	9	5	3	3	1	1	2	1	3	3/932	0·3
— —	...	128	1/280	118	86	32	21	7	8	2	1	1	2	...	1	3	1/690	0·1
Total...	243	4/970	8/1848	8/1502	11/1054	5/354	3/279	98	2/98	35	32	21	1/22	1/8	9	2/17	45/6530	0·6
Percentage death rate.	...	0·4	0·4	0·5	1·04	1·4	1·0	...	2·0	...	...	...	4·5	12·5	...	11·7	0·6	...

TABLE VII.

Classification according to number of wounds and lateness of arrival.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Institute.

Days late.	Number of wounds.																Total.	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
0 ...	4	3	3	...	2	...	...	...	...	...	1	...	...	...	...	...	13	...
1 ...	4	5	5	1	2	3	...	...	...	1	...	...	...	...	...	...	21	...
2 ...	7	2	3	2	...	1	...	...	...	...	...	...	...	...	...	...	15	...
3 ...	21	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	23	...
4 ...	28	...	1	...	1	...	...	...	...	...	...	...	...	...	...	...	30	...
5 ...	7	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	9	...
6 ...	4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4	...
7 ...	3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3	...
8 to 14 ...	2	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3	...
15 to 21 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
22 to 28 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
29 to 35 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
36 to 42 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
43 to 49 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
50 and over...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total ...	80	13	14	3	5	4	...	...	...	1	1	...	...	...	...	...	121	...
Percentage death rate. ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VII-a.

Classification according to number of wounds and lateness of arrival.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Centres.

Days late.	Number of wounds.																Total	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
0 ...	71	10	5	2	1	...	...	...	1	...	...	...	...	...	...	...	90	...
1 ...	31	6	4	4	4	3	...	...	...	...	...	...	...	...	...	...	52	...
2 ...	25	3	5	...	1	...	1	...	...	...	...	...	...	...	...	1	36	...
3 ...	7	1	2	...	...	1	1	1	...	...	...	...	...	...	...	...	13	...
4 ...	22	1	3	1	...	...	...	...	1	...	...	...	...	...	...	...	28	...
5 ...	7	1	...	1	...	...	...	...	...	..	...	...	...	...	...	...	9	...
6 ...	6	2	1	...	...	...	...	...	...	...	...	...	...	...	...	...	9	...
7 ...	3	...	2	1	..	...	...	...	...	...	...	...	...	...	...	...	6	...
8 to 14 ...	3	5	...	3	...	...	...	...	...	...	...	...	...	...	...	...	11	...
15 to 21 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
22 to 28 ...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...
29 to 35 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
36 to 42 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
43 to 49 ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
50 and over ...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Total ...	176	29	22	12	6	4	2	1	2	...	...	...	...	...	...	1	255	...
Percentage death rate.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VII-b.

Classification according to number of wounds and lateness of arrival.

Numerators=Deaths. Asiatics. *Denominators*=Cases.

Institute.

Days late.	Number of wounds.																Total.	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.		
0 ...	1	69	170	82	107	43	26	1/20	9	5	3	1/7	1	1	1/1	1	3/546	0·54
1 ...	6	206	434	228	223	86	38	32	11	8	6	5	...	...	4	3	1295	...
2 ...	1	63	135	52	39	13	23	6	3	...	2	...	...	...	1	...	344	...
3 ...	1	28	48	40	24	9	4	5	1	...	...	...	...	...	...	...	160	...
4 ...	29	35	42	17	20	1	3	3	1	...	1	...	...	...	...	...	152	...
5 ...	5	16	40	17	15	5	2	1	...	...	...	...	...	...	...	...	101	...
6 ...	10	10	15	4	8	...	2	...	2	...	...	...	...	...	...	...	51	...
7 ...	5	4	14	9	2	...	...	2	...	...	...	...	...	...	...	...	36	...
8 to 14 ...	3	24	52	10	14	1	3	...	...	...	...	...	...	...	...	...	107	...
15 to 21 ...	...	9	21	3	6	2	1	...	...	...	...	...	...	...	...	...	42	...
22 to 28 ...	...	...	7	...	1	...	...	...	...	...	...	...	...	...	...	...	8	...
29 to 35 ...	1	8	12	1	4	...	...	...	...	...	...	...	...	...	...	...	26	...
36 to 42 ...	...	1	...	...	...	...	...	1	...	...	...	...	...	...	...	...	2	...
43 to 49 ...	...	1	1	...	2	...	1	...	...	...	...	...	...	...	...	...	5	...
50 and over ...	...	2	11	2	2	...	...	...	...	...	...	...	...	...	...	...	17	...
Total ...	68	476	1002	465	472	160	103	1/70	27	13	12	1/12	1	1	1/6	4	3/2892	0·1
Percentage death rate.	...	...	...	...	...	...	...	1·4	...	...	...	8·3	...	...	16·6	...	0·1	...

TABLE VII-c.

Classification according to number of wounds and lateness of arrival.

Numerators=Deaths. Asiatics. *Denominators*=Cases.

Centres.

Days late.	Number of wounds.															Total.	Percentage death rate.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over.		
0 ...	12	59	3/136	90	70	33	12	3	7	2	3	3	...	...	1	2	3/433	0·6
1 ...	87	1/208	408	2/399	4/206	3/80	72	25	23	10	8	4	2	1	2	2	10/1537	0·6
2 ...	22	1/178	1/382	2/304	2/208	49	45	18	24	6	6	3	1/8	...	1	1/6	8/1260	0·6
3 ...	21	181	263	2/287	150	1/60	2/58	15	20	7	8	5	5	3	1	1	5/1085	0·4
4 ...	36	2/102	2/175	2/120	3/152	38	39	13	13	3	4	2	3	1/3	2	1/3	11/708	1·5
5 ...	12	48	103	68	59	31	16	2	1/5	3	1	...	3	1	...	2	1/354	0·2
6 ...	10	39	65	39	1/50	11	1/17	2	1	...	1	1	1	...	...	1	2/238	0·8
7 ...	3	22	47	28	19	10	2	7	4	1	...	...	...	...	...	...	143	...
8 to 14 ...	22	62	1/177	103	95	1/23	13	11	1/1	3	1	3	...	...	2	...	3/516	0·5
15 to 21 ...	3	31	52	34	1/17	18	2	...	...	...	...	...	...	...	...	...	1/157	0·6
22 to 28 ...	2	8	11	5	5	...	...	...	...	...	...	...	...	...	...	...	31	...
29 to 35 ...	6	13	6	15	12	1	...	2	...	...	...	...	...	...	...	...	55	...
36 to 42 ...	...	5	1/8	1	3	...	1	...	...	...	...	...	...	...	...	...	1/18	5·5
43 to 49 ...	2	7	7	3	3	...	...	...	...	...	...	...	...	...	...	...	22	...
50 and over ...	5	7	8	6	5	...	2	...	...	...	...	...	...	...	...	...	33	...
Total ...	243	4/970	8/1848	8/1502	11/1054	5/354	3/279	98	2/98	35	32	21	1/22	1/8	9	2/17	45/6590	0·6
Percentage death rate.	...	0·4	0·4	0·5	1·04	1·4	1·0	...	2·0	...	...	...	4·5	12·5	...	11·7	0·6	...

TABLE VIII.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Institute.

Deep	Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
+	+ ...	4	...	8	1	...	...	13	...
+	— ...	4	...	1	...	...	...	5	...
—	+ ...	3	...	95	1	...	...	99	...
—	— ...	4	...	...	...	...	...	4	...
Total ...		15	...	104	2	...	...	121	...
Percentage death rate.		...	...	...	...	...	...	...	...

TABLE VIII-a.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Centres.

Deep	Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
+	+ ...	10	1	12	...	1	...	24	...
+	— ...	6	1	5	...	...	...	12	...
—	+ ...	15	1	191	...	1	...	208	...
—	— ...	8	1	2	...	...	...	11	...
Total ...		39	4	210	...	2	...	255	...
Percentage death rate.		...	...	...	...	...	...	...	...

TABLE VIII-b.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators = Deaths. Asiatics. *Denominators* = Cases.

Institute.

Deep Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
+ + ...	1/1166	9	518	54	1/54	1/13	3/1814	0·16
+ − ...	489	28	36	5	...	...	558	...
− + ...	198	1	180	4	19	1	403	...
− − ...	103	6	8	...	...	...	117	...
Total ...	1/1956	44	742	63	1/73	1/14	3/2892	0·1
Percentage death rate.	0·05	...	...	...	1·3	7·1	0·1	...

TABLE VIII-c.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators = Deaths. Asiatics. *Denominators* = Cases.

Centres.

Deep Bare.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Precentage death rate.
+ + ...	12/2135	2/118	8/987	3/205	9/187	3/100	37/3732	0·9
+ − ...	897	78	3/137	42	1/13	9	4/1176	0·3
− + ...	347	13	1/563	1/24	38	1/7	3/992	0·3
− − ...	1/511	41	102	28	8	...	1/690	0·1
Total ...	13/3890	2/250	12/1789	4/299	10/246	4/116	45/6590	0·6
ercentage death rate.	0·3	0·8	0·6	1·3	4·0	3·4	0·6	...

TABLE IX.

Classification according to position of wounds and lateness of arrival.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Institute.

Days late.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0 ...	6	...	6	1	...	...	13	...
1 ...	4	...	16	1	...	...	21	..
2 ...	2	...	13	...	...	...	15	...
3 ...	1	...	22	...	...	...	23	...
4 ...	2	...	28	...	...	...	30	...
5 ...	...	...	9	...	...	...	9	...
6 ...	...	...	4	...	...	...	4	...
7 ...	...	...	3	...	...	...	3	...
8 to 14 ...	...	...	3	...	...	...	3	...
15 to 21 ...	...	...	...	...	...	...	...	...
22 to 28 ...	...	...	...	...	...	...	...	...
29 to 35 ...	...	...	...	...	...	...	...	...
36 to 42 ...	...	...	...	...	...	...	...	...
43 to 49 ...	...	...	...	...	...	...	...	..
50 and over .	...	...	...	...	...	...	...	...
Total ...	15	...	104	2	...	...	121	...
Percentage death rate.	...	...	...	...	...	...	...	...

TABLE IX-(*a*).

Classification according to position of wounds and lateness of arrival.

Numerators = Deaths. Europeans. *Denominators* = Cases.

Centres.

Days late.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0 ...	13	...	76	...	1	...	90	...
1 ...	9	1	41	...	1	...	52	...
2 ...	6	...	30	...	...	...	36	..
3 ...	2	1	10	...	...	...	13	...
4 ...	2	1	25	...	...	...	28	...
5 ...	2	...	7	...	...	...	9	...
6 ...	1	1	7	...	...	...	9	...
7 ...	1	...	5	...	...	...	6	...
8 to 14 ...	3	...	8	...	...	...	11	...
15 to 21 ...	...	...	...	...	...	...	...	...
22 to 28 ...	...	...	...	...	...	...	...	...
29 to 35 ..	...	...	1	...	...	...	1	...
36 to 42 ...	...	...	...	...	...	...	...	...
43 to 49 ...	...	...	...	...	...	...	...	...
50 and over .	...	...	...	...	...	...	...	...
Total ...	39	4	210	...	2	...	255	...
Percentage death rate.	...	...	...	...	...	...	...	...

TABLE IX-(*b*).

Classification according to position of wounds and lateness of arrival.

Numerators = Deaths. *Denominators* = Cases.

Asiatics.

Institute.

Days late.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0 ...	1/379	8	115	21	1/16	1/7	3/546	0·54
1 ...	897	23	310	24	37	4	1295	...
2 ...	222	5	101	8	7	1	344	...
3 ...	102	4	43	6	4	1	160	...
4 ...	81	1	64	2	3	1	152	...
5 ...	69	2	29	...	1	...	101	...
6 ...	31	...	17	1	2	...	51	...
7 ...	20	...	14	1	1	...	36	...
8 to 14 ...	78	...	27	...	2	...	107	...
15 to 21 ...	28	1	13	...	...	...	42	...
22 to 28 ...	6	...	2	...	...	...	8	...
29 to 35 ...	24	...	2	...	...	...	26	...
36 to 42 ...	2	...	...	...	...	...	2	...
43 to 49 ...	4	...	1	...	...	...	5	...
50 and over ...	13	...	4	...	...	...	17	...
Total ...	1/1956	44	742	63	1/73	1/14	3/2892	0·1
Percentage death rate.	0·05	...	...	...	1·3	7·1	...	...

TABLE IX-(*c*).

Classification according to position of wounds and lateness of arrival.

Numerators = Deaths. *Denominators* = Cases.

Asiatics.

Centres.

Days late.	Leg.	Trunk.	Arm.	Multiple.	Face.	Multiple face.	Total.	Percentage death rate.
0 ...	1/245	12	2/140	15	14	7	3/433	0·6
1 ...	2/923	1/71	4/392	71	3/58	22	10/1537	0·6
2 ...	2/742	1/41	1/313	1/75	2/64	1/25	8/1260	0·6
3 ...	3/631	39	301	1/55	35	1/24	5/1085	0·4
4 ...	4/424	32	3/181	1/26	3/28	17	11/708	1·5
5 ...	203	21	105	11	10	1/4	1/354	0·2
6 ...	127	10	77	11	1/8	1/5	2/238	0·8
7 ...	96	5	35	3	2	2	143	...
8 to 14 ...	313	5	1/153	1/23	1/18	4	3/516	0·5
15 to 21 ...	96	2	1/47	3	6	3	1/157	0·6
22 to 28 ...	18	2	10	...	1	...	31	...
29 to 35 ...	32	1	18	1	1	2	55	...
36 to 42 ...	1/7	4	5	1	...	1	1/18	5·5
43 to 49 ...	14	1	3	3	1	...	22	...
50 and over ...	19	4	9	1	...	...	33	...
Total ...	13/3890	2/250	12/1789	4/299	10/246	4/116	45/6590	0·6
Percentage death rate.	0·3	0·8	0·6	1·3	4·0	3·4	0·6	...

TABLE X.

Deaths from Hydrophobia amongst persons treated in

Europeans.

Institute.

Serial Number.	Case Number.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and position of wounds.						Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
					Kind.	Class.	Leg.	Trunk.	Arm.	Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.			Type.	Duration in days.

TABLE X-b.

Deaths from Hydrophobia amongst persons treated in

Asiatics.

Institute.

Serial Number.	Case No.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and position of wounds.					Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
					Kind.	Class.	Leg.	Trunk.	Arm.	Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
							Died during treatment.														
1	1283	10	M.	52	Dog ...	C	...	...	6	...	6	+	−	+	+	20	2	32	III	5 % 5cc.	12 days
							Died within 15 days after treatment.														
1	2350	22	M.	5	Dog ...	C	...	...	...	11	11	+	−	+	+	0	1	23	IVF	„ 7cc.	14 „
							Died after 15 days after treatment.														
1	2401	...	M.	4	Dog ...	D	5	...	...	9	14	+	−	+	+	0	2	34	IVF	„ 7cc.	14 days
2	2232	...	M.	4	Do. ...	C	7	...	...	...	7	+	+	+	+	0	4	143	IVE	„ 5cc.	„ „

TABLE X-c.

TABLE

Deaths from Hydrophobia

Asia

Cen

Serial No.	Case No.	Number of days from commencement of treatment to onset of symptoms.	Sex.	Age in years.	Biting animal.		Number and of wo		
					Kind.	Class.	Leg.	Trunk.	Arm.
								Died during	
1	414	11	F.	6	Dog ...	C	...	...	...
							Died within 15 days		
1	123	14	M.	65	Jackal ...	C	...	...	4
2	70	17	F.	½	Do. ...	C	...	...	...
3	546	17	M.	24	Dog ...	C	...	...	..
4	218	20	M.	10	Do. ...	C	...	...	...
5	7	20	F.	9	Do. ...	D	...	...	...
6	7	24	M.	9	Do. ...	D	...	...	2
7	2	25	M.	11	Do. ...	C	...	...	2
8	791	27	F.	65	Jackal ...	C	..	3	...
							Died after 15 days		
1	29	28	F.	30	Jackal ..	C	...	...	5
2	128	29	F.	30	Do. ...	C	...	...	6
3	132	29	M.	7	Dog ...	B	...	7	1
4	236	30	M.	60	Jackal ...	C	...	...	...
5	291	31	M.	10	Dog ...	C	4	...	...
6	153	33	M.	25	Do. ...	B	...	...	...
7	10	35	M.	9	Do. ...	C	5	...	...
8	135	35	M.	60	Jackal ...	C	...	...	...
9	87	36	F.	40	Dog ...	C	4	...	...
10	718	37	F.	10	Do. ...	C	3	...	3
11	140	37	M.	12	Do. ...	C	2	...	...
12	48	38	F.	10	Do. ...	C	...	...	2
13	66	39	M.	7	Do. ...	C	...	...	2
14	547	40	F.	6	Do. ...	C	...	...	4
15	53	42	M.	4	Do. ...	C	...	...	...
16	565	46	M.	40	Do. ...	C	5	...	...
17	11	46	M.	25	Do. ...	C	18	...	...

X.c.

amongst persons treated in

tics.

tres.

position unds.		Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
treat	*ment.*										Days.
2	2	+	—	+	—	11	1	23	IVf	5 %	7 cc. 11
after	*completio*	*n of*	*treat*	*ment.*							
...	4	+	—	+	+	20	2	36	IVe	5 %	7 cc. 14
13	13	+	—	+	+	4	1	22	IVf	,,	5 ,, 14
3	3	+	—	+	+	2	3	22	IVf	,,	10 ,, 14
1	1	+	+	+	—	2	2	24	IVf	,,	7 ,, 14
2	2	+	+	+	—	4	2	26	IVf	,,	7 ,, 14
...	2	—	—	+	—	0	2	26	III	,,	3 ,, 14
...	2	+	—	+	—	0	4	29	IVe	,,	5 ,, 14
...	3	+	—	+	+	1	2	30	III	,,	5 ,, 14
after	*treatment.*										
...	5	+	—	+	+	1	4	33	III	5 %	5 cc. 14
2	8	+	—	+	+	5	2	36	IVf	,,	10 ,, 14
...	8	+	—	+	+	8	2	39	III	,,	3 ,, 14
4	4	+	—	+	+	4	3	39	IVf	,,	10 ,, 14
...	4	+	—	+	+	4	2	37	III	,,	3·5 ,, 14
2	2	+	—	+	+	8	2	43	IVf	,,	10 ,, 14
...	5	+	—	+	+	3	2	40	IVe	,,	5 ,, 14
1	1	+	—	+	+	4	3	42	IVf	,,	10 ,, 14
...	4	+	+	+	—	1	3	40	III	,,	5 ,, 14
...	6	+	—	+	+	3	2	42	III	,,	3·5 ,, 14
...	2	+	—	+	+	4	3	44	IVe	,,	5 ,, 14
4	6	+	—	+	+	3	2	43	IVf	,,	10 ,, 14
1	3	—	—	+	+	2	2	43	II	,,	2 ,, 14
...	4	+	—	—	+	1	3	44	III	,,	3·5 ,, 14
4	4	+	+	—	—	1	2	45	IVf	,,	7 ,, 14
...	5	+	—	+	+	1	3	50	IVe	,,	7 ,, 14
...	18	+	—	+	+	2	3	51	IV	,,	7 ,, 14

TABLE

Sorial No.	Case No.	Number of days from commencement of treatment to onset of symptoms.	Sex	Age in years.	Biting animal.		Number and of wo		
					Kind.	Class.	Leg.	Trunk.	Arm.
							Died	*after*	*15 days*
18	708	46	M.	30	Dog ...	C	...	...	1
19	70	47	M.	6	Do. ...	C	3	...	...
20	117	49	F.	65	Jackal ...	C	3	...	2
21	505	50	M.	10	Dog ...	C	...	..	...
22	45	55	M.	6	Do. ...	C	2	...	2
23	528	64	M.	25	Do. ...	C	...	...	3
24	226	65	M.	12	Do. ...	C	...	...	3
25	9	66	M.	5	Do. ...	C	6	...	13
26	714	68	F	7	Do. ...	C	...	...	4
27	151	76	M.	20	Do. ...	D	...	...	5
28	31	78	F.	50	Do. ...	C	...	...	4
29	51	86	M.	25	Do. ..	C	...	...	12
30	162	86	M.	14	Do. ...	B	3	...	...
31	57	89	F.	8	Do. ...	C	4	...	...
32	69	95	M.	36	Jackal ...	C	3	...	...
33	39	101	F.	30	Do. ...	C	2	...	...
34	46	108	M.	40	Dog ...	C	4	...	...
35	84	113	F.	50	Do. ...	D	2	...	...
36	224	126	M.	10	Do. ...	C	...	...	...
37	112	317	M.	7	Do. ...	C	...	2	...

X-c—*contd.*

position unds.		Wounds.				Number of days late.	Duration of illness in days.	Interval between bite and death in days.	Class of cases.	Treatment.	
Face.	Total.	Deep.	Extensive.	Bare skin.	Uncauterised.					Type.	Duration in days.
after treatment—continued.											Days.
...	1	+	+	—	+	4	3	53	IVe	5 %	7cc. 14
...	3	+	+	+	—	3	2	52	IV	„	5 „ 14
1	6	+	+	+	+	5	3	58	IVf	„	10 „ 14
5	5	+	+	+	+	1	3	54	IVf	„	7 „ 14
...	4	+	—	+	+	2	3	60	III	„	3 „ 14
...	3	+	—	+	+	1	4	72	IV	„	7 „ 14
...	3	+	—	+	+	1	2	68	IV	„	5 „ 14
...	19	+	+	+	—	4	2	72	III	„	3·5 „ 14
...	4	+	+	+	+	4	3	75	III	„	3·5 „ 14
...	5	+	+	+	—	8	3	87	III	„	5 „ 14
...	4	+	—	+	+	1	3	82	IV	„	7 „ 14
...	12	+	—	—	—	2	2	90	III	„	5 „ 14
...	3	+	—	+	+	4	2	92	III	„	5 „ 14
...	4	+	+	+	+	2	3	94	III	„	5 „ 14
...	3	+	—	+	+	3	3	101	III	„	5 „ 14
...	2	+	—	+	+	36	2	139	III	„	5 „ 14
...	4	+	—	+	+	4	2	114	III	„	5 „ 14
...	2	+	—	—	—	0	2	115	II	„	2 „ 14
1	1	+	+	+	+	1	2	129	IVf	„	7 „ 14
...	2	+	—	+	+	2	3	322	III	„	3·5 „ 14

TABLE

Classification of the animal which bit,

Numerators = Deaths.

Animal.	Institute.								
	Number treated.			Percentage to total.			Death rate per cent.		
	Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.
Dog ...	118	3/2,785	3/2,903	97·5	96·3	96·3	...	0·1	0·1
Jackal ...	...	6	6	...	0·2	0·1	...	...	...
Cat ...	1	43	44	0·8	1·1	1·1	...	...	...
Monkey ...	2	40	42	1·7	1·3	1·3	...	...	...
Fox ...	...	.....	... ..	...	...	...	...	...	...
Man ...	...	11	11	...	0·3	0·3	...	...	...
Donkey ...	...	.....	.....	...	...	...	...	...	...
Horse ...	...	6	6	...	0·2	0·1	...	...	...
Buffaloes ...	...	.....	.....	...	...	...	...	...	...
Hyena ...	...	.....	.....	...	...	...	...	...	...
Wolf ...	...	.....	.....	...	...	...	...	...	...
Cow ...	...	.....	.....	...	...	...	...	...	...
Goat ...	...	.....	.....	...	...	...	...	...	...
Leopard ...	...	.....	.....	...	...	...	...	...	...
Pig ...	...	1	1	...	...	...	...	...	...
Mangoose ...	...	.....	.....	...	...	...	...	...	...
Camel ...	...	.....	.....	...	...	...	...	...	...
Calf ...	...	.....	.....	...	...	...	...	...	...
Panther ...	...	.....	.....	...	...	...	...	...	...
Ox ...	...	.....	... ..	...	...	...	...	...	...
Bear ...	...	.....	.....	...	...	...	...	...	...
Total ...	121	3/2,892	3/3,013	...	...	...	...	...	...

XI.

scratched or licked the patient.

Denominators = Cases.

Centres.								
Number treated.			Percentage to total.			Death rate per cent.		
Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.	Europeans.	Asiatics.	Combined.
248	35/5,796	35/6,044	97·4	87·9	88·2	...	0·6	0·5
3	10/670	10/673	1·1	10·1	9·8	...	1·4	1·4
4	21	25	1·5	0·3	0·3	...	...	...
...	14	14	...	0·2	.....	...	...	...
...	10	10	...	0·1	.....	...	...	...
...	20	20	...	0·3	.....	...	...	...
...	17	17	...	0·2	.....	...	...	...
...	13	13	...	0·1	.....	...	...	...
...	3	3	...	0·04	.....	...	...	...
...	2	2	...	0·03	.....	...	...	...
...	6	6	...	0·09	.....	...	...	...
...	2	2	...	0·03	.....	...	...	...
...	1	1	...	.....	.....	...	...	...
...	1	1	...	.....	.....	...	...	...
...	.....	.....	...	.....	.....	...	...	...
...	2	2	...	0·03	.....	...	...	...
...	1	1	...	.....	.....	...	...	...
...	2	2	...	0·03	.....	...	...	...
...	2	2	...	0·03	.....	...	...	...
...	4	4	...	0·06	.....	...	...	...
...	3	3	...	0·04	.....	...	...	...
255	45/6,590	45/6,845	...	.....	.....	...	...	...

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TABLE XII.

Number of patients treated by months.

	Institute.			Centres.			Institute and Centres combined.		
	Europeans	Asiatics.	Total.	Europeans.	Asiatics.	Total.	Europeans.	Asiatics.	Total.
January ...	7	251	258	23	525	548	30	776	806
February ...	13	270	283	9	446	455	22	716	738
March ...	6	297	303	19	611	630	25	908	933
April ...	3	277	280	32	538	590	35	815	850
May ...	4	236	240	5	601	606	9	837	846
June ...	20	236	256	7	499	506	27	735	762
July ...	15	209	224	22	548	570	37	757	794
August ...	27	236	263	45	512	557	72	748	820
September ...	5	244	249	14	626	640	19	870	889
October ...	1	190	191	10	527	537	11	717	728
November ...	12	192	204	28	523	551	40	715	755
December ...	8	254	262	41	634	675	49	888	937
Total ...	121	2,892	3,013	255	6,590	6,865	376	9,482	9,858

TABLE XIII.

Statement showing brains submitted and examined for rabies.

1937.

Serial No.	Animal.	Number of brains examined.	Positive.	Negative.	Unfit for examination.
1	Dogs	237	189	44	4
2	Goat	1	1	...	...
3	Jackals	2	2	...	...
4	Buffaloes	1	1	...	...
5	Cows	1	1	...	...
6	Horse	1	...	1	...
7	Donkey	1	...	1	...
	Total ...	244	194	46	4

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